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MECHANISMS OF MERCURY DEPOSITION UNDER BOREAL FOREST CANOPIES: CONTRIBUTIONS OF NEW AND RECYCLED INPUTS

by

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in

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Mechanisms of Mercury Deposition Under Boreal Forest Canopies: Contributions of New and Recycled Inputs** submitted by **Jennifer Anne Graydon** in partial fulfillment of the requirements for the degree of Master of Science in Environmental Biology and Ecology.

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Abstract

Dropping of senescent foliage (litterfall) represents the largest flux of mercury to boreal forest soils, and understanding the source(s) of this Hg (new atmospheric vs. recycled inputs) is crucial for a complete and accurate Hg mass-balance budgets. Stomatal uptake of atmospheric gaseous elemental Hg (Hg(0)), root uptake of soil Hg, and retention of ionic Hg (Hg(II)) from wet deposition were evaluated as Hg input pathways for boreal forest canopies at the Experimental Lakes Area (ELA, northwestern Ontario). Mechanistic experiments were conducted using a new dynamic chamber for measuring Hg(0) exchange from foliage, and stable Hg isotope tracers. Results were consistent with the general concensus that most of the Hg in litterfall is derived from stomatal uptake of Hg(0), and not from root uptake of soil Hg. Some of the Hg in litterfall may be derived from long term retention of Hg(II) from wet deposition.

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Chapter 1 Mechanisms of mercury deposition under boreal forest canopies

General Introduction

Mercury (Hg) is a toxic and persistent pollutant that affects the nervous system of humans and other vertebrates (Dellinger *et al.*, 1995). In fact, the number of fish and wildlife Hg advisories in Canada and the U.S. has been steadily increasing (EPA Fact Sheet, 2001). Monomethylmercury (MMHg) is the most toxic form of Hg and a common contaminant of fish in Canada (Ontario Ministry of the Environment, 1999) due to the ability of MMHg to bioaccumulate in organisms and biomagnify through food webs. Consumption of contaminated fish is the most significant route of exposure to environmental Hg for humans (Inskip and Piotrowski, 1985). It is generally thought that emissions of Hg from human activities such as coal burning have led to the increase in advisories.

Recent studies have demonstrated the importance of Hg exchange between the atmosphere and biosphere, and have shown that this exchange is more dynamic than previously hypothesized (Lindberg *et al.*, 1998, Gustin *et al.*, 1999, Poissant *et al.*, 2000, Mason *et al.*, 2000, St. Louis *et al.*, 2001). Hg is a highly volatile metal, and as a result, a global pollutant. Most (~98%) of the Hg in the atmosphere is elemental Hg vapour, Hg^0 (Hg(0)), which is easily transported and widely dispersed to remote ecosystems due to its relatively long atmospheric residence time of approximately one year (Mason *et al.*, 1994). Hg(0) is only weakly soluble in water, and therefore must undergo oxidation to dissolve in precipitation. The oxidized form of Hg is ionic Hg^{2+} (Hg(II)), and atmospheric deposition is the dominant source of Hg(II) to most water bodies and terrestrial environments (Bloom and Watras, 1989, Swain *et al.*, 1992, Fitzgerald *et al.*, 1998), even in remote Boreal ecoregions (e.g., Schroeder *et al.*, 1998). The metal enters lakes through direct deposition on the lake

surface and in runoff, and bacteria present in anaerobic environments such as lake sediments and wetlands methylate Hg(II) to MMHg (Rudd, 1995, Hurley *et al.*, 1995, Baldi, 1997). This MMHg is then available to bioaccumulate and biomagnify through aquatic food webs or, alternatively, it can undergo demethylation (Verta *et al.*, 1994). In addition, inorganic Hg(II) and MMHg can also be transformed photochemically (e.g., Sellers *et al.*, 1996, Amyot *et al.*, 1997) or possibly biologically (Mason *et al.*, 1994) to elemental Hg(0) and lost to the atmosphere. The amount of "re-emission" of Hg to the atmosphere influences the residence time of Hg in aquatic and terrestrial pools, and hence the amount of Hg subsequently available for methylation.

One important knowledge gap in the understanding of how Hg moves from the atmosphere, through watersheds and into fish involves the role of plants in the deposition of Hg. Plants are a major component in the internal cycling of Hg in watersheds and may act as conduits of atmospheric Hg into the terrestrial system. Plant foliage can take up "new" atmospheric mercury by numerous mechanisms including wet deposition of Hg in precipitation (Figure 1.1). Dry foliar surfaces are also dynamic exchange surfaces and can function either as a source or sink for Hg (Lindberg, 1996). Dry deposition onto foliage can occur as particulate Hg, as an aerosol, and possibly as direct adsorption of reactive Hg forms (Iverfeldt, 1991). In addition, atmospheric Hg(0) is taken up through stomata. However, not all Hg deposition may be considered new input, as Hg(0) released from soils below the canopy through volatilization may also be taken up by stomata, and soil-water Hg is absorbed by roots and incorporated into plant tissues (Lindberg *et al.*, 1979). Currently, root uptake of soil Hg (Lindberg *et al.*, 1979) and foliar uptake of Hg in wet deposition (Iverfeldt, 1991) are not considered major pathways of Hg accumulation in plants, whereas uptake of

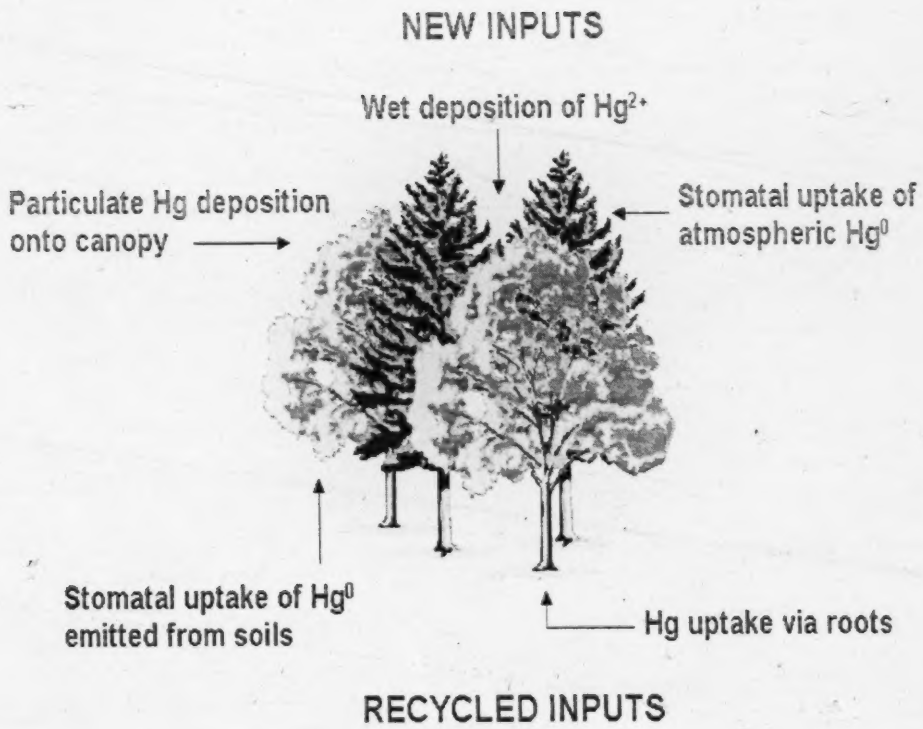
NEW INPUTS
Wet deposition of Hg^{2+}
Particulate Hg deposition onto canopy
Stomatal uptake of atmospheric Hg^0
Stomatal uptake of Hg^0 emitted from soils
Hg uptake via roots
RECYCLED INPUTS

Figure 1.1 Recycled (green) and new atmospheric (blue) Hg input pathways to canopy foliage (modified after St. Louis *et al.*, 2001).

atmospheric Hg(0) through stomata may represent a significant accumulation pathway (Lindberg, 1996).

Following leaf drop, litter decomposes and releases various elements, and in this capacity contributes significant fluxes of Hg to the forest floor (St.Louis *et al.*, 2001). As plant tissues decompose, mercury is released into the soil where it can be bound to organic matter and/or carried into nearby lakes in runoff. In the Boreal ecoregion, St. Louis *et al.* (2001) recently showed that the forest canopy was an important contributor to fluxes of MMHg and total Hg (THg) to watersheds. For example, annual fluxes of MMHg and THg in throughfall (wet deposition that passes through the forest canopy) plus litterfall at the ELA were ~ 2 and 3 times greater than fluxes of MMHg (0.1 ug/m^2) and THg (7 ug/m^2) in wet deposition over open areas such as lakes. Almost all the increased flux of Hg under the forest canopy occurred as litterfall, and as a result, it is important to understand where the Hg in foliage originates. Because plant foliage provides an excellent surface for photochemical reduction of deposited Hg(II) (e.g., Lindberg *et al.*, 1998), any Hg remaining in the forest canopy or on ground vegetation following precipitation events can be photoreduced to Hg(0) and fluxed back to the atmosphere, affecting *net* deposition of Hg to watersheds. For this reason, the role of the canopy in intercepting "new" atmospheric Hg and recycling the soil pool must be clarified for a complete understanding of the effects of reduced atmospheric emissions on watersheds.

To examine the relationship between atmospheric Hg deposition and MMHg accumulation in fish, a whole-ecosystem loading experiment using stable Hg isotopes is being carried out at the Experimental Lakes Area (ELA, northwestern Ontario). The *Mercury Experiment to Assess Atmospheric Loading In Canada and the United States* (METAALICUS) simulates, in a controlled manner, the impact of Hg contaminated deposition on

biogeochemical processes leading to elevated MMHg concentrations in fish (Hintelmann *et al.*, 2002). Different mercury isotopes have been loaded on to the upland, wetland, and the lake surface of a single watershed to determine how route of entry affects Hg accumulation in fish.

The stable isotopes of Hg and their percentage natural abundances (in parentheses) are as follows: ^{196}Hg (0.15), ^{198}Hg (10.92), ^{199}Hg (16.84), ^{200}Hg (23.12), ^{201}Hg (13.22), ^{202}Hg(29.80), and ^{204}Hg (6.85). At these natural abundances, this assemblage of isotopes represents the "native" Hg in the environment. Using individual purified stable Hg isotopes as environmental tracers allows experimentally deposited Hg to be discriminated from background native Hg already in watershed pools. Isotopic Hg is then used to follow Hg through transformations in the Hg biogeochemical cycle and through different compartments of the ecosystem (e.g. soil, vegetation, water) over time (Hintelmann *et al.*, 2002). Attempts to determine the effect of atmospheric Hg loading on fish MMHg concentrations without using isotopic tracers have been confounded by the numerous interconnected pathways and transformations in the Hg biogeochemical cycle. This experiment provides the control necessary to examine the direct relationship between new atmospheric loading of Hg on the bioaccumulation of MMHg in fish, and the timing of this response.

Within the framework of the METAALICUS project, the goal of this thesis is to examine mechanisms of Hg deposition under boreal forest canopies using a novel stable isotope-loading approach. This thesis consists of two research chapters. The first (Chapter 2) presents the design and calibration of a new dynamic flux chamber for measuring Hg(0) exchange from canopy foliage. The second chapter (Chapter 3) presents the results of several experiments designed to examine the mechanisms of stomatal uptake of Hg(0), root

uptake of soil-Hg, and wet deposition of Hg(II) as contributors to Hg accumulation in leaves and litterfall. Finally, a concluding chapter (Chapter 4) relates these mechanistic studies to the overall METAALICUS whole-ecosystem project and identifies possible avenues of future research.

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Chapter 2 Design and calibration of a new dynamic chamber for measuring Hg(0) exchange from canopy foliage

Introduction

Mercury (Hg) is a highly volatile metal, and as a result, a global pollutant (Lindqvist, 1985). Atmospheric Hg is known to interact with terrestrial ecosystems, however there is little information available concerning the transfer of Hg across the atmosphere-terrestrial interface (Kim *et al.*, 1997). Atmospheric deposition is the dominant source of Hg(II) to most water bodies and terrestrial environments (Lindqvist, 1985). Once deposited, inorganic Hg(II) can be methylated to monomethylmercury (MMHg; the organic, toxic form of Hg) in anaerobic environments such as lake sediments and wetlands, and is then available to bioaccumulate through food webs (Baldi, 1997). In addition, inorganic Hg(II) and MMHg can also be transformed photochemically or possibly biologically to elemental Hg(0) and lost to the atmosphere (Amyot *et al.*, 1994, Amyot *et al.*, 1997, Carpi and Lindberg, 1997, Morel *et al.*, 1998, Lalonde *et al.*, 2002). The amount of "re-emission" of Hg to the atmosphere influences the residence time of Hg in aquatic and terrestrial pools, and hence the amount of Hg subsequently available for methylation. Since few measurements of terrestrial Hg evasion have been made, the role of terrestrial landscapes as sources of Hg to the atmosphere are highly uncertain, and likely underestimated in global Hg budgets (Gustin *et al.*, 1996, Leonard *et al.*, 1998). Even fewer attempts have been made to quantify biogenic Hg emissions, for example from forest canopies, even though foliar surfaces typically represent 1-10 times the surface areas of subtending soils (Gustin *et al.*, 1996).

The two most common approaches used to measure gas exchange between the atmosphere and forest canopies are micrometeorological techniques (flux-gradient or eddy correlation) and enclosure "chamber" methods (Garcia *et al.*, 1990, Poissant *et al.*, 1999).

Micrometeorological techniques impose little disturbance on the plants under study and integrate gas exchange over a large area (Garcia *et al.*, 1990, Gustin *et al.*, 1999b), but do not allow for control and manipulation of environmental conditions in field mechanistic experiments. Enclosure methods utilize smaller plot sizes, making it possible to compare responses of enclosed vegetation to experimental treatments (Gustin *et al.*, 1999a). Enclosure systems are also generally less expensive and more easily transported from site to site than micrometeorological systems. However, the microclimate of vegetation enclosed in chambers is invariably altered from natural conditions, with the degree of alteration depending on the design of the chamber (Garcia *et al.*, 1990).

Both micrometeorological techniques and enclosure techniques have been used to measure exchange of Hg(0) at the terrestrial landscape/atmosphere interface, but most studies have been carried out in areas of natural Hg enrichment or anthropogenic Hg contamination (e.g., Lindberg *et al.*, 1992, Lindberg, 1996, Gustin *et al.*, 1999b, Gustin *et al.*, 1999c, Poissant *et al.*, 1999). The most common chamber design used to quantify gas exchange is an open system one, where there is a net flow of air through the chamber. This configuration is often used in chambers that measure Hg(0) exchange from natural surfaces like water, soil and vegetation (e.g., Xiao *et al.*, 1991, Kim and Lindberg, 1995, Wallschläger *et al.*, 1999). Here, Hg(0) flux is estimated from the product of air flow rate and the difference in Hg(0) concentration in air entering and exiting the chamber (Wallschläger *et al.*, 1999). It is extremely important that the environment within the chamber be as homogeneous as possible. For example, several studies have shown that low air turnover rate in dynamic chambers can result in underestimates of Hg(0) emissions from soils, due to the effects on gradients of Hg(0) at the soil surface boundary layer (Wallschläger *et al.*, 1999, Gillis and Miller, 2000). Similarly, there is evidence that at least part of the Hg(0) exchange at the

leaf/atmosphere interface is governed by gradients of Hg(0), and for this reason, fluxes may be underestimated if chambers interfere with turbulent flow at the surface of leaves. There is also significant evidence that foliar exchange of Hg(0) occurs via stomata (Du and Fang, 1982, Lindberg *et al.*, 1992, Lindberg 1996). It is also therefore critical that chamber designs do not interfere with normal physiological responses (e.g., photosynthesis & transpiration) of enclosed foliage.

Furthermore, materials used in the construction of chambers to measure gas exchange in general can have a profound influence on the effectiveness of the system, and hence should be considered carefully (Bloom *et al.*, 1980, Dixon and Grace, 1982, Long *et al.*, 1996, Garcia *et al.*, 1990). This is especially true for chambers designed to measure Hg(0) exchange, due to ease of contamination with Hg(0) from some chamber components. In addition to acting as a potential source of Hg(0), some chamber materials may also adsorb/absorb Hg(0), or block spectral wavelengths important for the photochemical reduction of Hg(II) to Hg(0).

In this study we describe a chamber we designed for measuring Hg(0) exchange from foliage undergoing normal physiological functioning. We used a stable ^{202}Hg isotope tracer to calibrate the chamber's ability to measure fluxes of ^{202}Hg(0) from foliar surfaces following simulated atmospheric deposition of ^{202}Hg(II). Volatilization rates of ^{202}Hg(0) measured using the chamber were compared to the decline in foliage concentrations of ^{202}Hg(II) directly. We also examined the effects of ambient radiation conditions (presence of visible and UV wavebands) on the photochemical reduction of ^{202}Hg(II) and subsequent loss of ^{202}Hg from foliar surfaces.

Methods

Gas exchange system

To measure Hg(0) fluxes from canopy vegetation, an open chamber system in single-pass configuration was designed and constructed (Figure 2.1). The chamber was built from materials with low Hg, water, and carbon dioxide (CO_2) absorption/adsorption properties. The body of the chamber consisted of an inflatable heat-sealed bag made of polyvinylidene chloride-coated polypropylene film (Propafilm-C; UCB films) (Figure 2.2). Propafilm-C has been widely used in photosynthetic measurement chambers due to its high thermal transmission, and low permeability to CO_2 and water (Parkinson, 1985, Garcia *et al.*, 1990). Inlet and outlet tubing attachments were Teflon PFA straight compression fittings mounted into threaded holes in a flat, oval piece of 6.35 mm thick Teflon PTFE sheeting. A plastic DC brushless computer fan (77mm, 0.75 m^3 min^{-1}) for mixing the air within the chamber was mounted on the Teflon sheet using nylon screws. The chamber film was then sealed around the oval Teflon sheet using a large rubber O-ring that stretched tightly into the grooved edge of the sheet. All tubing was PTFE (6.35 mm O.D., 4.76 mm I.D.). Air was pumped into the chamber with two DC brushless pumps (Brailsford TD-4X2NA, 10 L min^{-1} capacity), to create positive pressure within the chamber (Figure 2.1). Maintaining positive pressure within a well-mixed chamber ensures that any leaks are outward and that air, Hg(0), H_2O and CO_2 concentrations are not affected if leakage occurs. Air flow into the system was maintained between 9-12 L min^{-1}. Total air flow into the chamber was measured by a stainless steel thermal mass flow meter (GFM17S, 0-30 L/min capacity; Aalborg Instruments) at the chamber inlet. The chamber was large enough (approximately 10 L) to contain substantial foliage necessary for a measurable Hg(0) flux, yet small enough to ensure

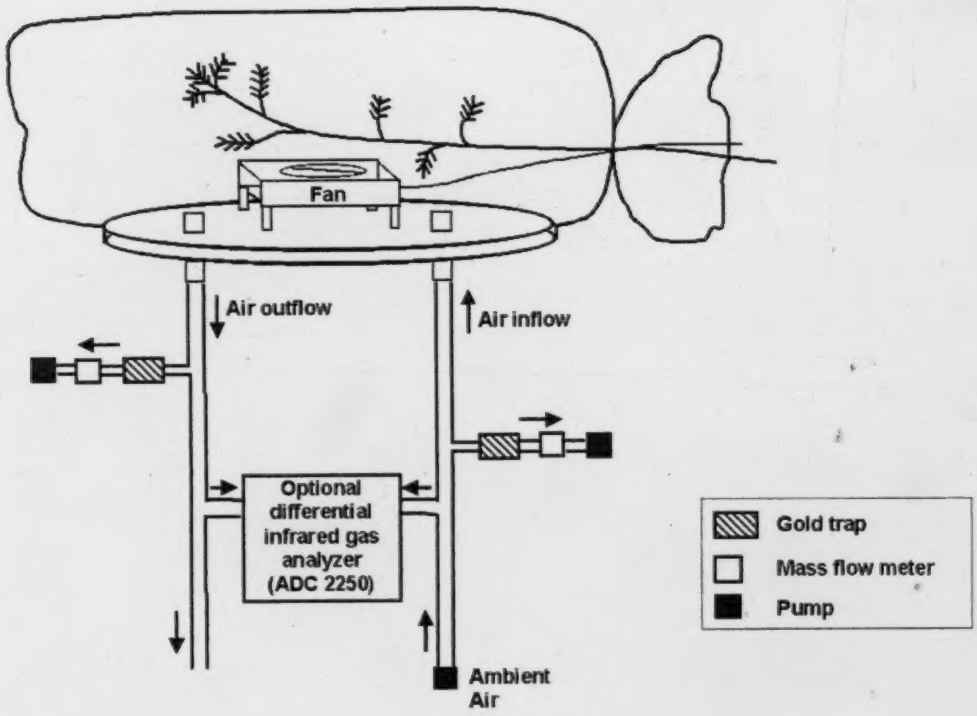

Figure 2.1 Diagram of new dynamic open chamber to measure Hg(0) exchange from canopy foliage.

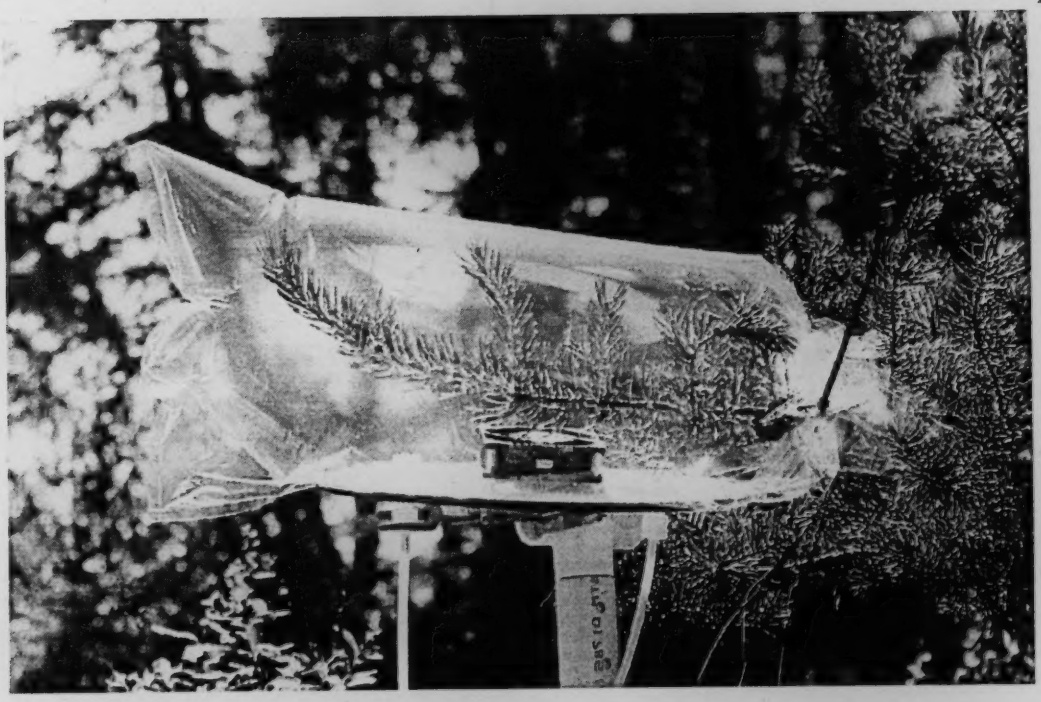

Figure 2.2 Photograph of new dynamic open chamber to measure Hg(0) exchange from canopy foliage (photo by Vince St.Louis, 2001).

thorough mixing of the air within and leaf-flutter. The branch under study was placed inside the chamber, and the bag was tightened around the branch using cable ties.

Concentrations of Hg(0) were measured on sub-samples of air entering and leaving the chamber. Small air pumps (Brailsford TD-2NA, 0-5 L min^{-1} capacity) sub-sampled inlet and outlet lines through gold-coated glass bead traps that stripped Hg(0) completely from the air. Thermal mass flow meters (GFM17, 0-5 L min^{-1} capacity, Aalborg Instruments) and totalizers (TOT10, Aalborg Instruments) measured instantaneous and total air flow through the gold traps, respectively. Soda lime traps were used upstream, in line with the gold traps, to remove humidity from the sub-sampled air. Comparing Hg(0) recovered at the chamber outlet relative to the inlet determined direction and rate of the foliar flux. For runs where only native Hg was of interest (e.g., blank chamber runs for system validation), Hg(0) was thermally desorbed from the traps at 400 °C into an ultra-high purity argon carrier gas, and detected by a cold-vapour atomic fluorescence spectrometry (CVAFS) Hg detector (Tekran model 2500). Star Chromatography Workstation software (Varian) was used for peak integration. For experiments using isotopic Hg(0), detection was by using inductively coupled plasma mass spectrometry (ICP-MS; Finnigan MAT, Model Element 2) instead of CVAFS. Isotope analysis was performed at the Trent University isotopic Hg laboratory. For both ambient and isotopic Hg(0), fluxes were calculated using the following equation:

$$J_{Hg(0)} = \frac{(Hg(0)_o/\mu_o)-(Hg(0)_e/\mu_e)\times(\mu_m)}{wt.}$$

Where: $J_{Hg(0)}$ = flux of isotopic or ambient Hg(0)(ng g^{-1} hr^{-1}); $Hg(0)_o$ = total isotopic or ambient Hg(0)on outlet gold trap (ng); μ_o = total air flow through outlet gold trap (L); $Hg(0)_e$ = total isotopic or ambient Hg(0)on inlet gold trap (ng); μ_e = total air flow through inlet gold trap (L); μ_m = main line flow rate (L h^{-1}); wt = total freeze-dried branch weight (g).

System validation

Chamber blanks were obtained using an empty chamber and outdoor air on numerous occasions throughout the spring/summer of 2001. Blank chamber runs were 30 minutes in length. On each occasion that chamber blanks were run in replicate, concentrations of Hg(0) in air entering and leaving the chamber were compared using a Wilcoxon signed rank test. A mass balance approach was also used to blank the chamber. We introduced $^{198}Hg(0)$ into the inlet line using a custom-built 3.7 cm long Teflon permeation tube (Vici-metronics, Santa Clara, CA, USA). The permeation tube was enclosed in a glass U-tube and submerged in a 30° C water bath. Air was passed over the permeation tube at a rate of 1 L min^{-1}, and fed into the chamber inlet air stream (8.4 L min^{-1}). Air entering and leaving the chamber was sub-sampled onto gold traps and analyzed as described above.

Gold traps were always thermally desorbed (cleaned) immediately prior to their use in the field, and blank chamber traps containing native Hg were analyzed within a few hours after the completion of runs. Traps containing isotopes were sealed using Teflon tape, individually bagged, and sealed in mason jars purged with ultra-high purity nitrogen to prevent scrubbing of atmospheric Hg(0) during transport.

Effects of the chamber on plant physiology (photosynthesis and transpiration)

Because factors which alter CO_2 assimilation also affect stomatal opening and Hg(0) exchange (Lindberg, 1992), it was important that our chamber did not alter normal leaf physiological function. We therefore modified the chamber in the laboratory to determine in a controlled manner if it affected apparent photosynthesis (APS) and transpiration (E) of enclosed vegetation. An infrared gas analyzer (IRGA; ADC 2250) was connected to the

system to sample air entering and leaving the chamber for concentrations of CO_2 and H_2O. CO_2 and H_2O exchange was estimated from the product of the airflow rate and the difference in CO_2 concentration of air entering and exiting the chamber. Photosynthetic and transpiration light response curves were generated for both a corn plant (*Zea mays*) and a loblolly pine (*Pinus taeda*) tree by incrementally moving a large artificial light source (250 Watt, H5/H37 Hg vapour lamp) closer to the plants. Concentrations of CO_2 and H_2O entering and leaving the chamber were logged by the IRGA every 10 seconds at each light level until the CO_2 concentrations leveled off and remained at a steady state for several minutes. At that point, the light level was increased and measurements continued. Photosynthetically active radiation (PAR) was measured using a Licor 190-SA quantum sensor and a universal voltage output amplifier (UTA, EME systems) and logged by the IRGA. In the run using corn, the PAR sensor was mounted on one side of the chamber only, and variation in light transmission to other areas of the chamber was not considered. In contrast, for the pine run, PAR readings were taken below, above, to both sides and in the center of the chamber once at each light level used before mounting on one side of the chamber. These readings from different parts of the chamber were averaged, and all other PAR measurements corrected for this variation to get an accurate estimate of total light actually reaching the enclosed foliage. Steady state rates of APS and E were calculated using equations published by Pearcy *et al.*, (1989):

$$APS = [(\mu_o c_o - \mu_e c_e)/L] \times 1\times10^6 \quad [\mu mol\ m^{-2}\ s^{-1}]$$

(where μ_e = air flow into chamber [mol s^{-1}]; μ_o = air flow leaving chamber [mol s^{-1}]; c_e = mol fraction CO_2 in air entering chamber with air at water content at which it passes through IRGA [mol CO_2 mol air^{-1}]; c_o = mol fraction CO_2 in air leaving chamber with air at water content at which it passes through IRGA [mol CO_2 mol air^{-1}]; L = single-sided leaf surface area for corn [m^2]or total leaf area for pine [m^2])

$$E = [(\mu_o w_o - \mu_e w_e)/L] \times 1\times10^6 \qquad [\mu mol\ m^{-2}\ s^{-1}]$$

(where μ_o = air flow leaving [mol air s^{-1}]; μ_e = air flow entering [mol air s^{-1}]; w_o = water leaving [mol H_2O mol air^{-1}]; w_e = water entering [mol H_2O mol air^{-1}]; L = single-sided leaf surface area for corn [m^2] or total leaf surface area for pine [m^2])

Since airflow is usually greater at the chamber outlet than at the inlet because transpiration increases the total volume, the flow at the chamber outlet is corrected as follows:

$$\mu_o = \mu_e(1-w_e / 1-w_o) \qquad [mol\ s^{-1}]$$

(where μ_e = air flow entering chamber [mol air s^{-1}]; μ_o = air flow leaving chamber [mol air s^{-1}];w_e = water entering [mol H_2O mol air^{-1}]; w_o = water leaving [mol H_2O mol air^{-1}])

For comparison of APS of foliage within the Hg flux chamber, an ADC PLC leaf chamber (for corn) or conifer chamber (for loblolly pine) and LCA-2 portable photosynthesis system was simultaneously attached to the plant under study (Figure 2.3). For the portable ADC Ps system, PAR was measured directly beside the chamber using a Licor sensor and light meter, and $\Delta[CO_2]$, RH, and PAR measurements were made only once at each light level, once steady state had been reached. APS was calculated as follows:

$$APS = [(\mu/L) \times \Delta[CO_2] \times 1.8] \times 1000 \qquad [mg\ CO_2\ dm^{-2}\ h^{-1}]$$

(where: μ = air flow rate over leaf surface (L h^{-1}); L = single-sided leaf area for corn (dm^2) or total leaf area for pine (dm^2); $\Delta[CO_2]$ = the difference in $[CO_2]$ between the inlet and outlet of the leaf chamber (L CO_2 L^{-1} air); 1.8 = density of CO_2 (g L^{-1}) used to convert the concentration of CO_2 from units of gas volume (L) to mass (mg); 1000 = # mg/g.)

The APS value obtained using the portable photosynthesis system was multiplied by 6.31 to convert to μmol CO_2 cm^{-2} s^{-1} to directly compare with APS measurements from the Hg flux chamber (Nobel, 1974

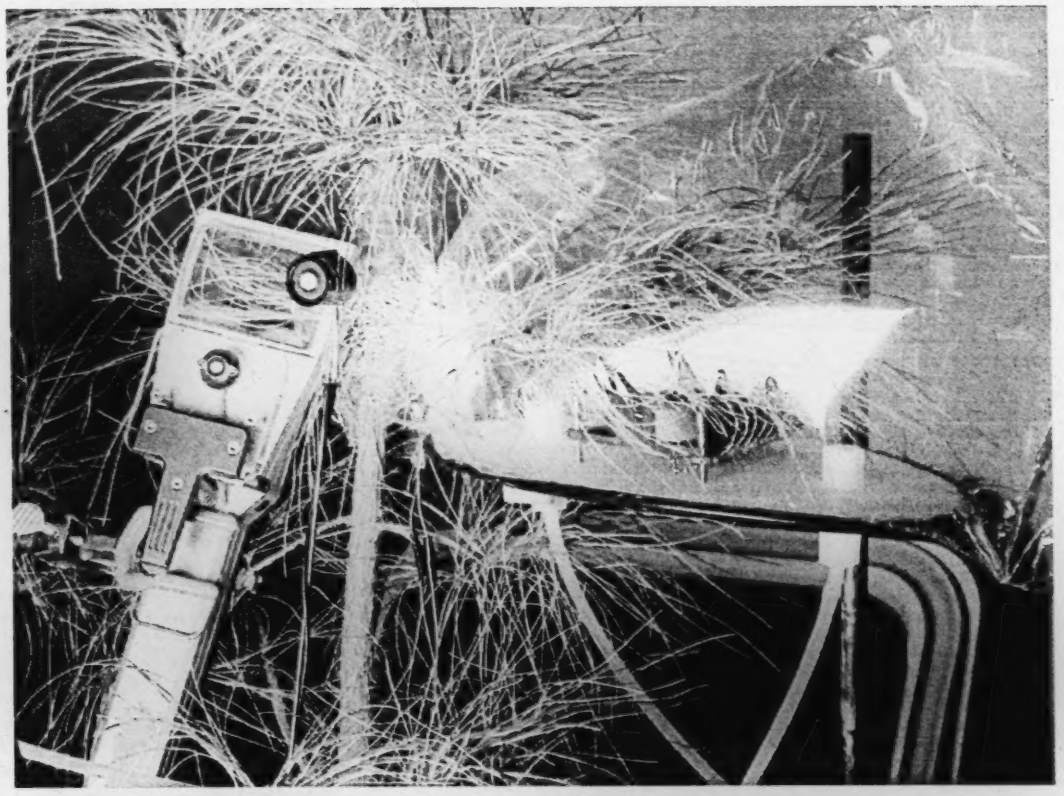

Figure 2.3 Laboratory intercomparison of loblolly pine APS and E rates using the ADC broadleaf chamber and the new open chamber for measuring Hg(0) exchange from canopy foliage.

Following runs, enclosed foliage was collected to determine single-sided leaf area using a computer scanner and digital imaging software (SigmaScan, Jandel Scientific). For pine needles, single-sided leaf area was doubled as an estimate of total leaf area.

Losses of Hg from foliage following simulated precipitation events: comparison of losses measured using the Hg flux chamber with decreases in foliage Hg concentration over time.

Year 2000

The goal of this experiment was to quantify retention of ionic Hg applied as simulated wet deposition on leaf surfaces over time, and how spike "binding time" prior to sun exposure and washing during rain events influence affected losses of Hg from foliage. Four small jack pine (*Pinus banksiana*) and birch (*Betula papyrifera*) trees were selected in an upland ridge top region at the Experimental Lakes Area (ELA, northwestern Ontario). A simulated wet deposition of Hg was prepared using 500 μL of primary spike solution of $^{202}HgCl_2$ (40 mg L^{-1} in concentrated HCl) mixed into 1 L of water in a hand-held plastic lawn and garden sprayer. Water for the spike was collected from a large oligotrophic lake (Winnange Lake) with low native Hg concentrations. The pH of the solution was adjusted to approximately 3.5 with 100 μL of 27 % NaOH. Lake water was selected as a matrix for all spike solutions used in these experiments to provide ligands (like those present in rain) to which the isotope could bind before application to the tree. The final ^{202}Hg concentration of the spike was 20 μg L^{-1} and the total mass of ^{202}Hg applied to the foliage was 20 μg in 1 L of water. This water volume was equivalent to a 1 mm rain event on 1 m^2 of ground surface area. Between 13:15 and 13:30 on 30 May 2000, isotope was applied to two of the jack pines (JP #1 & 2) and two of the birch trees (B # 1 & 2). The entire liter of solution was applied

so that foliage was dripping. Upon complete drying (approximately 15 minutes later), a sample of foliage was clipped using strict clean hands-dirty hands protocol (St.Louis *et al.*, 1996). Isotope was applied to the other two birch (B 3 & 4) and jack pine trees (JP 3 & 4) after sunset (between 20:50 and 21:05) in the same manner as earlier in the day. However, these trees were not sampled until early the next morning before sunrise (5:30). All eight trees were sampled at 15:30 that afternoon, and again on 2, 9 June, and 11 July. Two days after the afternoon spike application there was a 10 mm rain event.

Samples were freeze-dried and dry weights (leaves + branches for jack pine, and leaves only for birch) were measured. Samples were completely homogenized using stainless steel coffee grinders. Grinders were rinsed with Milli-Q water and cleaned with paper towels between samples to prevent cross-contamination.

100 mg sub-samples were digested in 20 mL glass vials using 10 mL of a 7:3 (vol/vol) mixture of concentrated nitric and sulfuric acid. Vials were covered with glass marbles and samples were heated on a hot plate at 80 °C under slow reflux until the production of brown nitrous gases ceased. Samples were diluted with water as needed to a desired concentration range of 0.1 - 1 ppb. Final sample volume was typically 10 mL. THg content of the digests was determined by continuous flow injection cold vapor generation with ICP/MS detection. The acidified samples were continuously mixed with a solution of stannous chloride (reductant) using peristaltic pumps. The gaseous Hg(0) formed was separated in a gas liquid separator (Model LI-2) and swept into the plasma of the ICP/MS (Hintelmann and Ogrinc, 2002, in press). Limits of detection (LOD) for the Hg isotopes in the samples depended on the concentration of native Hg present in the respective sample. Therefore, to detect an applied isotope with certainty, it must have been present at a concentration >0.5-1% of native Hg. The LOD varied with the precision of the isotope ratio

measurement during each run, which for these samples ranged from 0.2-1 ng/g (Hintelmann *et al.* 2002).

Year 2001

The tree spraying experiment was repeated in July 2001. In addition to quantifying total losses of isotope from foliage by following tissue concentrations over time, the Hg flux chamber was used to quantify the flux of $^{202}Hg(0)$ applied to the foliage. In 2000, we lowered the concentration of ^{202}Hg applied to a small jack pine to 400 ng L^{-1}, a level necessary to detect the isotope on the vegetation over time, but still much higher than concentrations actually measured in precipitation at the ELA (St.Louis *et al.* 1995). 20 µL of the same primary spike solution used in 2000 was mixed into 2L of Winnange Lake water. The total amount of isotope delivered to the tree was 0.8 µg m^{-2}, approximately a tenth of the annual deposition of 7 µg Hg m^{-2} at the ELA. Due to the extremely small amount of spike added, the pH of the solution was not adjusted for this application.

The jack pine was sprayed at 15:00 on 5 July 2001. The foliage was allowed to dry thoroughly for 2 hours prior to commencing flux measurements and sampling tissue. The selected branch was always enclosed in the chamber at least 15 minutes prior to attachment of gold traps. During this period of equilibration, the interior of the chamber was well mixed and the air exchange rate was maximal (9 - 10 L min^{-1}). The first two flux measurements following the spike application occurred at 2 and 5 hours post-spray. Between these two initial runs, the branch was removed from the chamber. All runs were 30 minutes in length. In subsequent measurements, the branch remained enclosed in the chamber at full mixing and flushing rates for the few minutes it took to replace gold traps between duplicate runs. Attempts were always made to use the same branch for all flux measurements. In total,

evasion of native and isotopic Hg(0) was measured at 2, 5, 24, 25, 46.5, 48.5, 97 and 97.5 hours post-spray. Gold traps were analyzed for isotopic and native Hg content by ICP/MS. PAR data were obtained from the ELA meteorological site located approximately 2 km away. PAR was measured using a LiCor Li190SA sensor and a LiCor Li-1000 logger, and values were integrated over a 10 min interval. The branches used in the flux chamber and all subsequent tree-spraying runs were harvested at the end of the last flux measurements. These branches were freeze-dried to obtain dry weights to which flux rates were normalized.

Tissue sampling occurred at approximately 2, 5, 25, 47, 97 hours and finally 34 days post-spray. Tissues were processed and analyzed as described for the 2000 experiment. Isotope loss rate estimates were calculated using the decline in tissue concentrations over time, and compared to the instantaneous fluxes estimated using the chamber.

Year 2002

The tree spraying flux chamber calibration experiment was repeated again in 2002. In this experiment, the same spike concentration of ^{202}Hg was used as in 2001 (0.4 $\mu g\ L^{-1}$), however both the total amount of isotope and water volume applied to the tree were half as much as the 2001 experiments. In 2002 a total of 0.4 μg ^{202}Hg was applied to the tree (a twentieth of annual Hg deposition per m^2 in the open) in 1 L of Winnange Lake water. Instead of using ambient PAR values from the meteorological station, PAR was measured directly outside the flux chamber during all flux runs using a Licor 190 SA quantum sensor. PAR values were logged every 10 seconds and averaged every minute by a Campbell CR10X datalogger.

A black spruce (*Picea mariana*) tree was selected for this last experiment. The tree was sprayed at 10:00 on 17 May 2002. A cloud-free day was selected to minimize interference by cloud cover with the ambient solar spectrum. A 20 minute blank chamber run was

performed while the foliage dried in the sun. Approximately one hour after the spray was applied, the first flux measurements (all 30 min runs) were conducted. In subsequent runs, film filters draped over the chamber were used to selectively remove certain wavelenghts from the ambient radiation spectrum to determine which were most important in driving photoreduction of ionic Hg to Hg(0) and evasion to the atmosphere. We first used Lee UV 260 film to block all UV-B and UV-A wavelengths from reaching the foliage inside the chamber (Figure 2.4). We used type-D Mylar to remove only the UV-B wavelengths in the next run. We then used a second layer of Propafilm-C as a control for the presence of a second layer of film on the chamber. For a final run, we removed all filters from the chamber. It was hypothesized that we would see a decrease in the flux of $^{202}Hg(0)$ as shorter, more energetic wavelengths were removed from the spectrum reaching the chamber. The branch was enclosed in the chamber continuouly for all filter runs at full flush rates (10 - 12 L min^{-1}). The branch from which fluxes were measured on day 1 was collected at the end of the last run, freeze-dried, and weighed. Tissue sampling from the spruce occurred at 3, 5.5, 27.5, 73.5, 122 and 460 hours post-spray. Gold traps and vegetation samples were processed as described above.

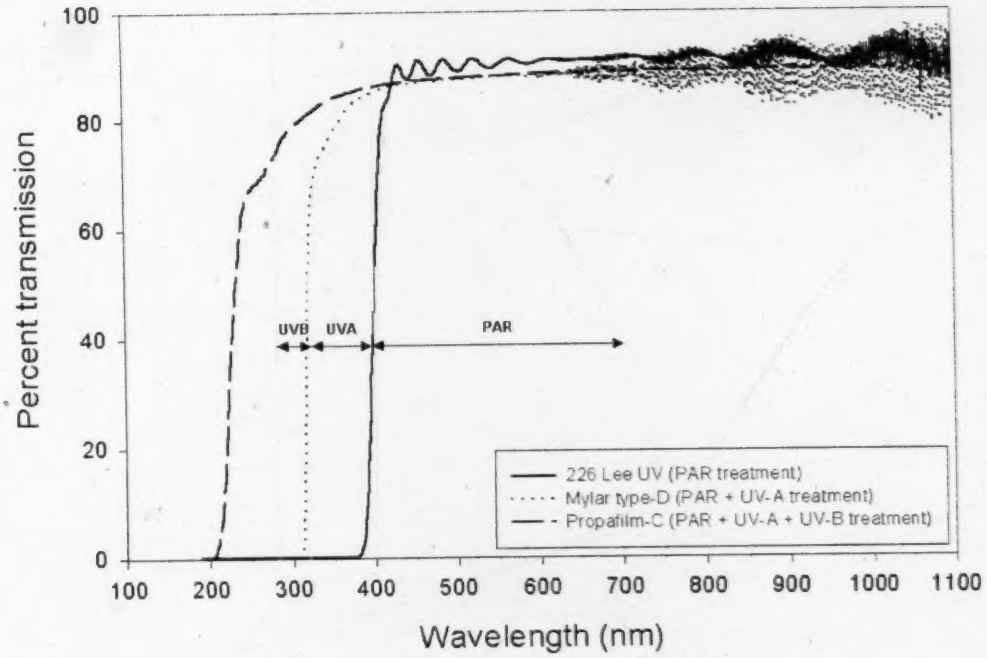

Figure 2.4 Percent transmission of infrared, visible and ultra-violet radiation wavebands by 226 Lee UV, Mylar type-D, and Propafilm-C light filters used in 2002 tree spraying experiments.

Results

System validation

In each series of chamber blanks, there was no significant difference between concentrations of Hg(0) entering and leaving the chamber (Wilcoxon signed rank test, $p<0.01$, $\alpha=0.05$). The mean difference in Hg(0) concentration entering and leaving the chamber (± standard error) for all runs combined was 0.13 (± 0.11) pg L^{-1}. In the mass-balance chamber blank using $^{198}Hg(0)$, the difference between $^{198}Hg(0)$ concentration of air entering and leaving the chamber was similar at 0.17 pg L^{-1}. These differences suggest that on average the chamber was a very small sink for Hg(0), negligible compared to Hg(0) fluxes reported from vegetation. Hence, flux measurements were not corrected for blanks.

Effects of chamber on plant physiology (photosynthesis and transpiration)

APS and E rates increased as the light was moved closer to the corn foliage enclosed in the Hg flux chamber (Figure 2.5). Measurements made with the ADC broadleaf chamber showed that foliage responded immediately to the increased light level with an increase in APS (not shown), whereas there was an obvious lag time in detection of APS changes in the larger Hg flux chamber. The lag effect in detection of physiological changes, and the reestablishment of equilibrium within the Hg flux chamber, can be seen when almost all light was instantaneously removed from the foliage with black plastic. Although APS and E values decreased, E rates did not reach a steady state before the end of the run. APS measurements of foliage enclosed in the Hg flux chamber were much lower than for foliage in the ADC broadleaf chamber at each light level (e.g., 5.8 vs. 22.5 μmol CO_2 m^{-2} s^{-1} at maximal irradiance in the Hg flux chamber and ADC chamber, respectively) (Figure 2.5).

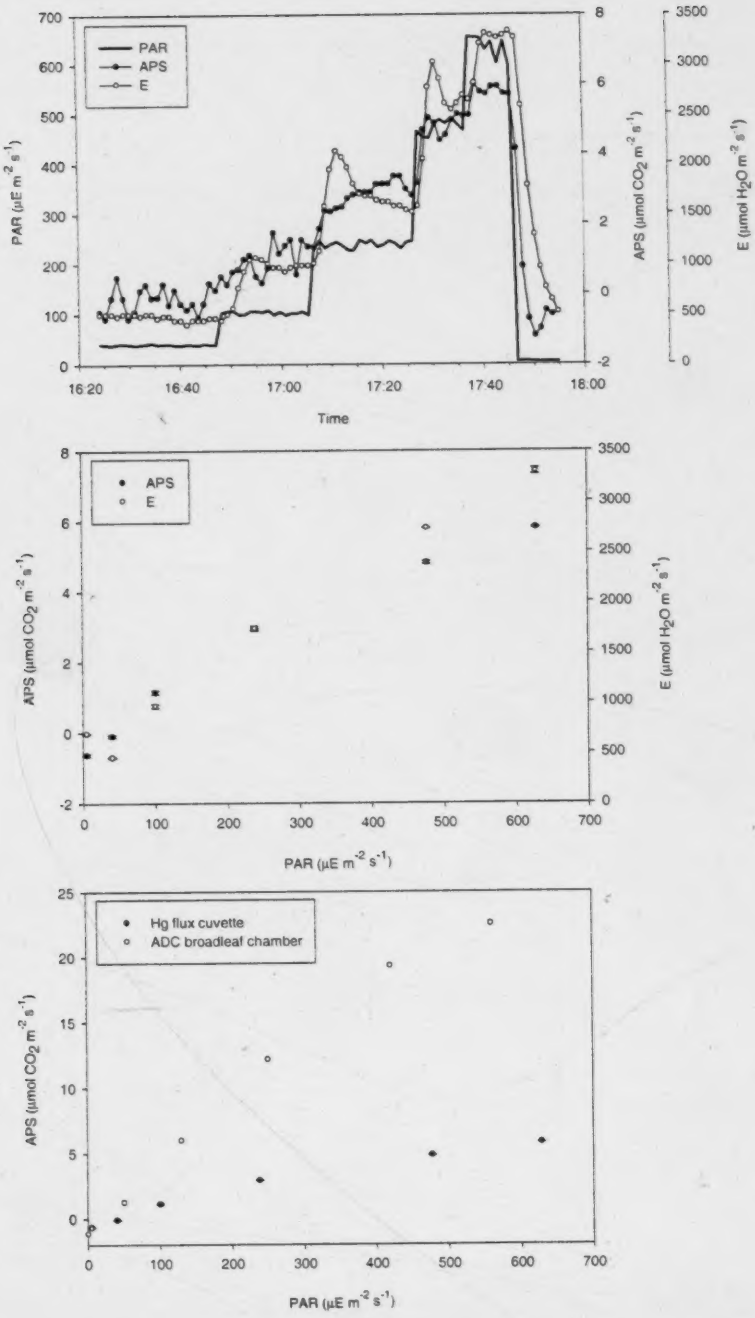

Figure 2.5 APS and E light response of corn in Hg flux chamber (top panel- time series, center panel- response curves) and chamber intercomparison using ADC broadleaf chamber and Hg flux chamber (bottom panel).

Slope of the light response curve and maximal photosynthetic rate of corn were both underestimated by the Hg flux chamber in this run.

Loblolly pine foliage also responded as expected to ambient PAR levels by showing increased APS and E rates as the light source was moved closer (Figure 2.6). Similar to the run using corn, however, detection of responses and reestablishment of equilibrium within the chamber after a change of light level was faster in the ADC chamber than in the Hg flux chamber. Again, E only declined slowly when light was blocked from the chamber (Figure 2.6). In contrast to the run using corn, the calculated rates of APS and E of foliage in both chambers were much more comparable. Slopes of the light response curves were similar, and leveling to maximal APS rates began to occur at around the same amount (1.5 μmol CO_2 m^{-2} s^{-1}).

Losses of Hg from foliage following simulated precipitation events: comparison of losses measured using the Hg flux chamber with decreases in foliage concentration over time

Year 2000

All trees showed a rapid initial loss of ^{202}Hg from foliage (10-40%) within 26 hours of spike application (Figure 2.7). By day 3 post-spray, 40-50% of the spike was gone from all trees, and after 42 days, >80% and >60% of ^{202}Hg applied to the birch and jack pine trees, respectively, was gone. Rain events (e.g., 10 mm 3 days post-spray) had no observable effect on the exponential loss of ^{202}Hg from the leaves. This was also evident in the gradual loss of spike between 9 June and 11 July despite 58 mm of rain. Although there may have been some initial difference between loss of isotope from trees sprayed in the afternoon versus those sprayed after sundown (especially for the birch trees), there was no significant

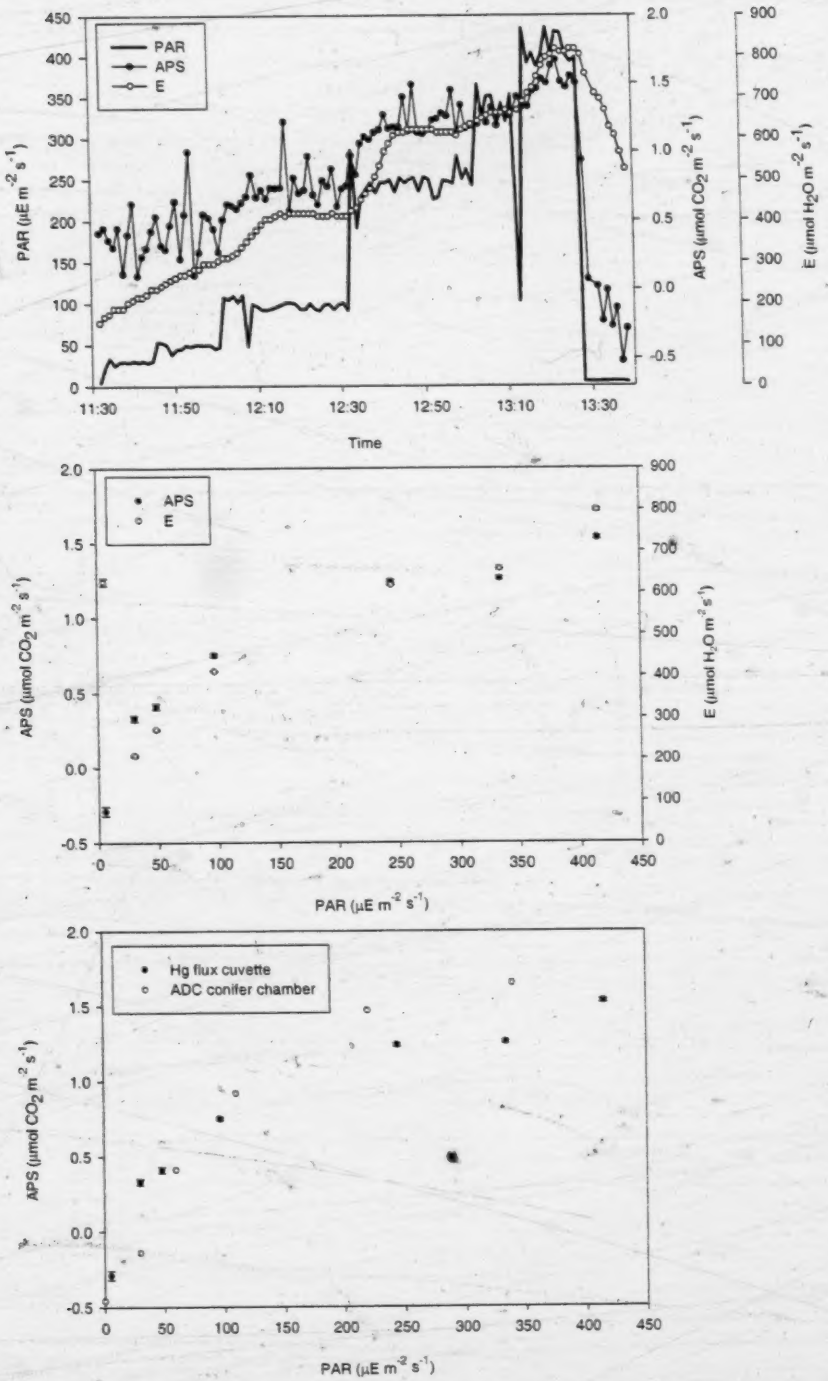

Figure 2.6 APS and E light response of loblolly pine in Hg flux chamber (top panel- time series, center panel- response curves) and chamber intercomparison using ADC conifer chamber and Hg flux chamber (bottom panel).

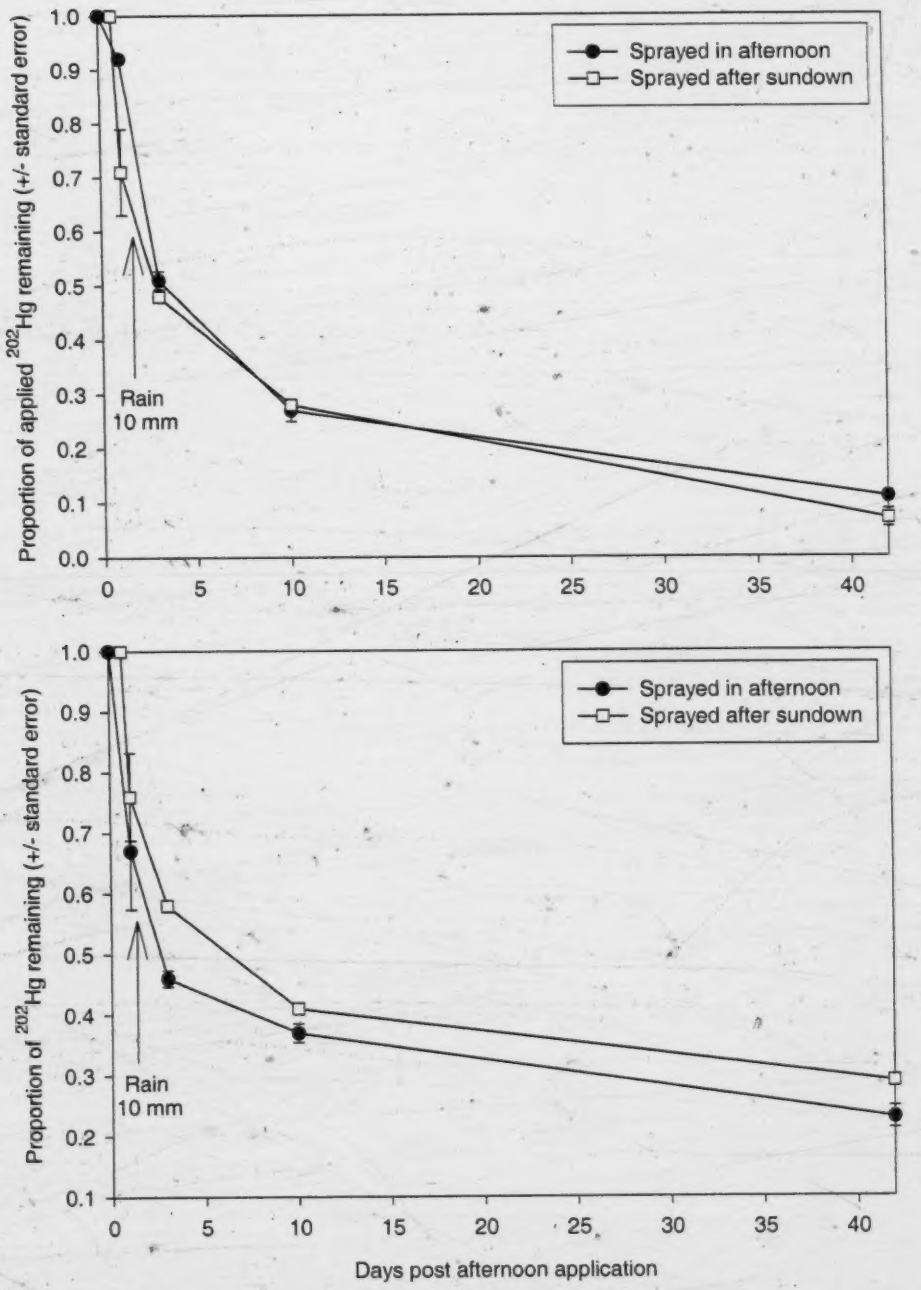

Figure 2.7: Average proportion of applied ^{202}Hg remaining on birch (top panel,) and jack pine (bottom panel) trees sprayed in the afternoon (n=2 for each species) and after sundown (n=2 for each species) and over time. The 10 mm rain event 3 days post spray is indicated.

difference between the proportion of isotope remaining on leaves of either species sprayed in the afternoon and after sundown by day 10 post-spray (repeated measures ANOVA, p=0.09; α=0.05) (Figure 2.7). Statistical results should not be considered reliable, however, because the data was not normally distributed with equal variances due to the small number of samples. Log transforming the data failed to correct this problem. Concentrations of ^{202}Hg in birch foliage were initially higher (35 - 44 ng g^{-1}) than in jack pine foliage (17 - 24 ng g^{-1}) (Figure 2.8), however jack pine foliage retained a slightly greater proportion of applied ^{202}Hg over the long term (20 - 30%) compared to birch foliage (10%). Concentrations of native Hg in all trees showed a slight increase over time (Figure 2.8).

Year 2001

Flux of ^{202}Hg(0) from jack pine foliage was highest immediately after spike application (2.5×10^{-3} ng g^{-1} hr^{-1}) (Figure 2.9). At 5 hours post-spray, ambient light had decreased considerably, and the flux of ^{202}Hg(0) also decreased to 5.7×10^{-4} ng g^{-1} hr^{-1}. The following day was overcast and rainy, and both measurements performed on this day showed no detectable ^{202}Hg(0) flux from sprayed foliage. However, the following several days were sunny, and detectable ^{202}Hg(0) fluxes were measured twice around 47 hours (average ± standard error=$7.9 \pm 1.3\times10^{-4}$ ng g^{-1} hr^{-1}) and twice around 97 hours post-spray ($6.9\times10^{-4} \pm 7\times10^{-6}$ ng g^{-1} hr^{-1}).

Native Hg(0) flux was approximately an order of magnitude greater than the flux of ^{202}Hg(0), and also highest in the first post-spray measurement (2.0×10^{-2} ng g^{-1} hr^{-1}) (Figure 2.9). Similar to the pattern observed for the flux of ^{202}Hg(0), native Hg(0) flux also decreased

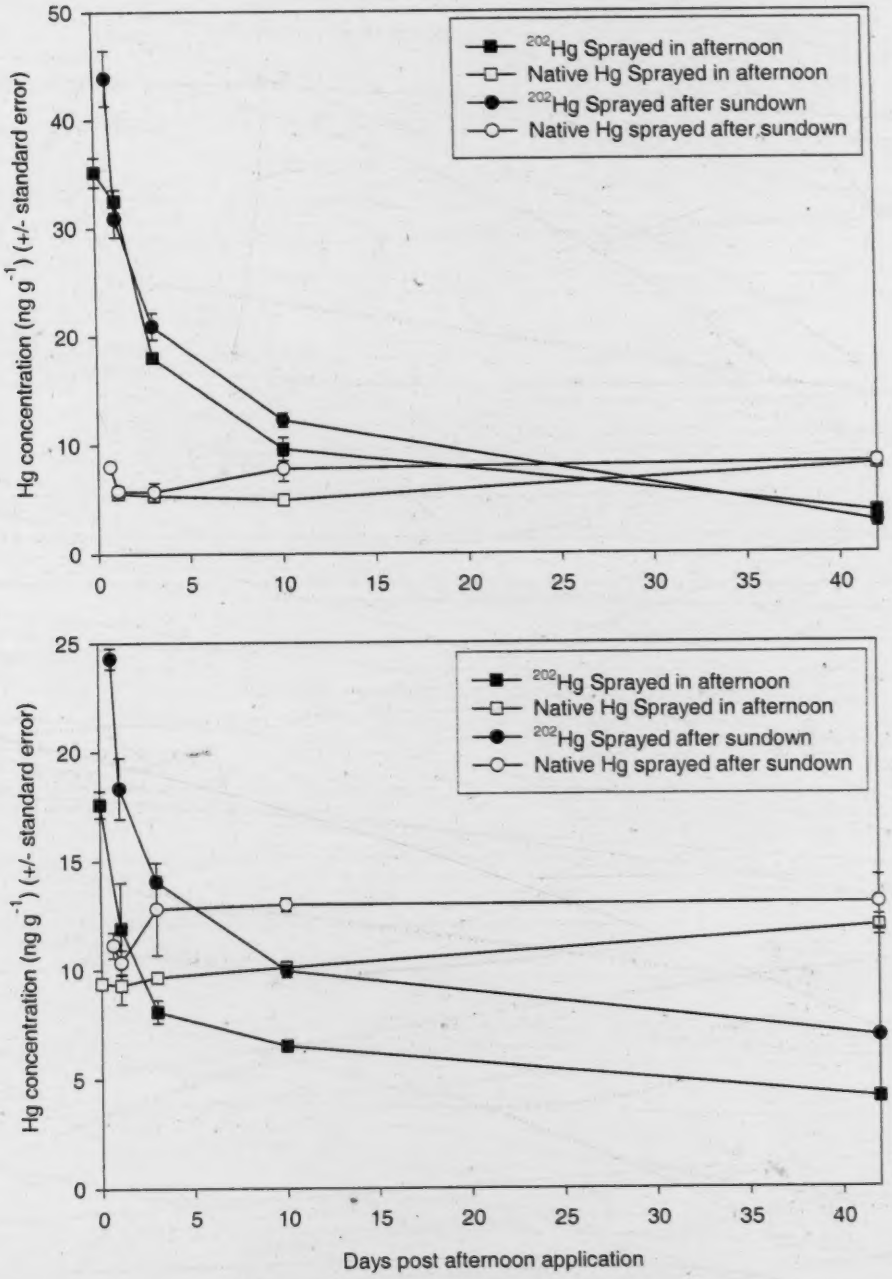
Hg concentration (ng g^{-1}) (+/- standard error)
^{202}Hg Sprayed in afternoon
Native Hg Sprayed in afternoon
^{202}Hg Sprayed after sundown
Native Hg sprayed after sundown
Days post afternoon application

Figure 2.8 Average concentrations of native Hg and ^{202}Hg in birch (top panel) and jack pine (bottom panel) sprayed in the afternoon (n=2 for each species) and after sundown (n=2 for each species) over time.

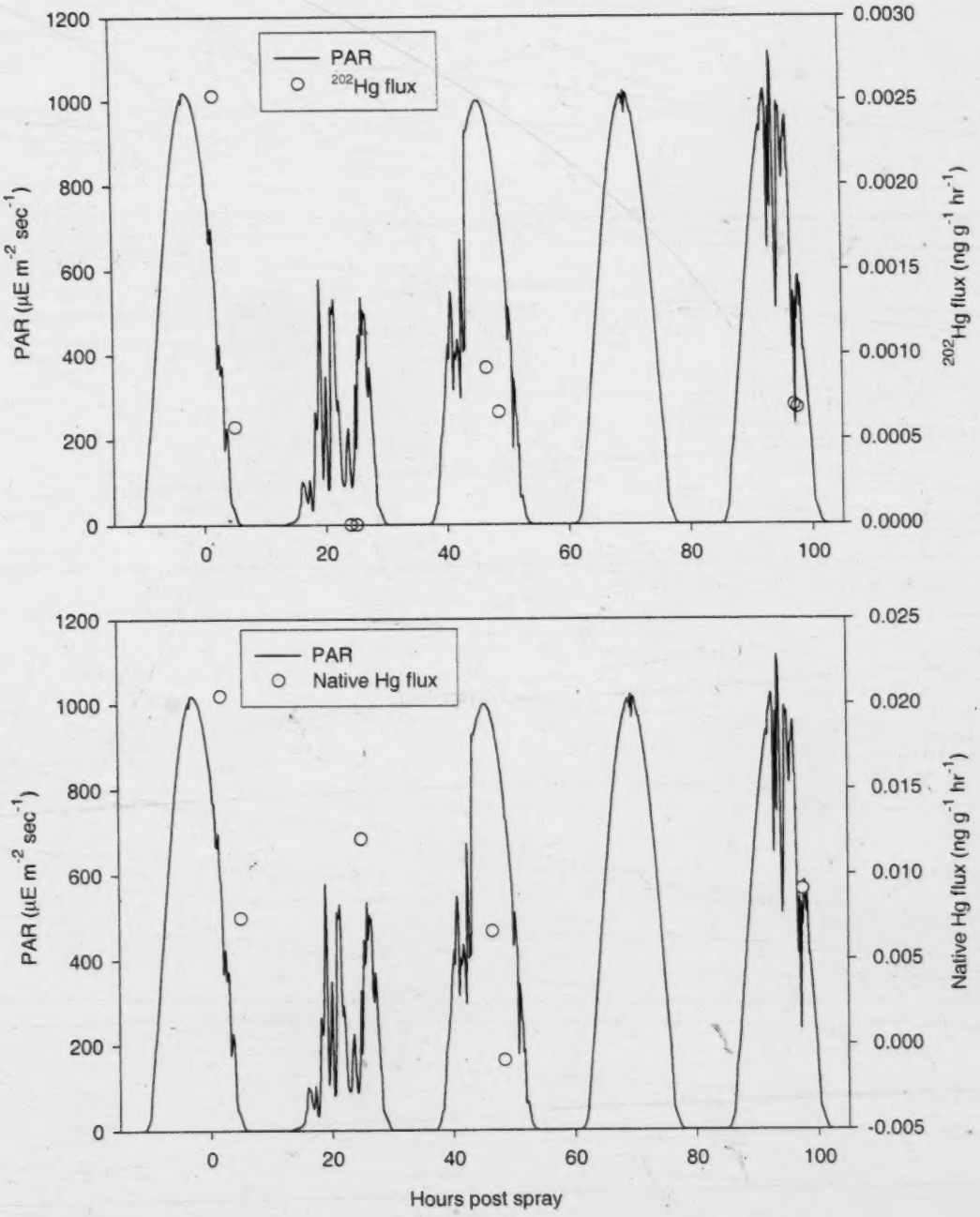

Figure 2.9 ^{202}Hg(0) (top panel) and native Hg(0) (bottom panel) fluxes with ambient PAR conditions from 5 July 2001 jack pine spray-tree experiment.

by 5 hours post-spray. However, unlike the spike, native Hg(0) flux from foliage was detectable on the following overcast day at 25 hours post-spray (1.2×10^{-2} ng g^{-1} hr^{-1}). For subsequent runs, native Hg(0) flux ranged between -8.8×10^{-4} (deposition) and 9.1×10^{-3} ng g^{-1} hr^{-1}.

Tissue ^{202}Hg concentrations on the 2001 spray trees tended to decrease over time (Figure 2.10), however, there was no initial exponential loss of ^{202}Hg from the foliage in 2001, possibly due to the much smaller amount of spike used. Native Hg concentrations in foliage again showed a slight increase over time.

Estimated rates of ^{202}Hg loss using tissue concentrations were higher than rates of loss estimated using instantaneous flux measurements. For example, between 5 hours and 25 hours post-spray, the estimated loss of spike using tissue concentrations was 3.00×10^{-3} ng g^{-1} hr^{-1}, whereas instantaneous flux measurements at these times were 0.574×10^{-3} and 0 ng g^{-1} hr^{-1}, respectively. Similarly, between 47 and 97 hours post-spray, loss rates based on tissue concentrations were 1.40×10^{-3} ng g^{-1} hr^{-1}, whereas instantaneous flux rates at these times were only 0.79×10^{-3} and 0.69×10^{-3} ng g^{-1} hr^{-1}, respectively.

Year 2002

PAR levels peaked and fell gradually over the course of the 2002 spray tree experiment (Figure 2.11). Even with this gradual decrease in PAR, all measurements were conducted under full sunlight with no disturbance by cloud cover (1590-1791 μE m^{-2} s^{-1}). Native Hg(0) fluxes in all runs were small and positive (0.019-0.036 ng g^{-1} hr^{-1}), Presence or absence of UV filters had no obvious effect on the size or direction of native Hg flux.

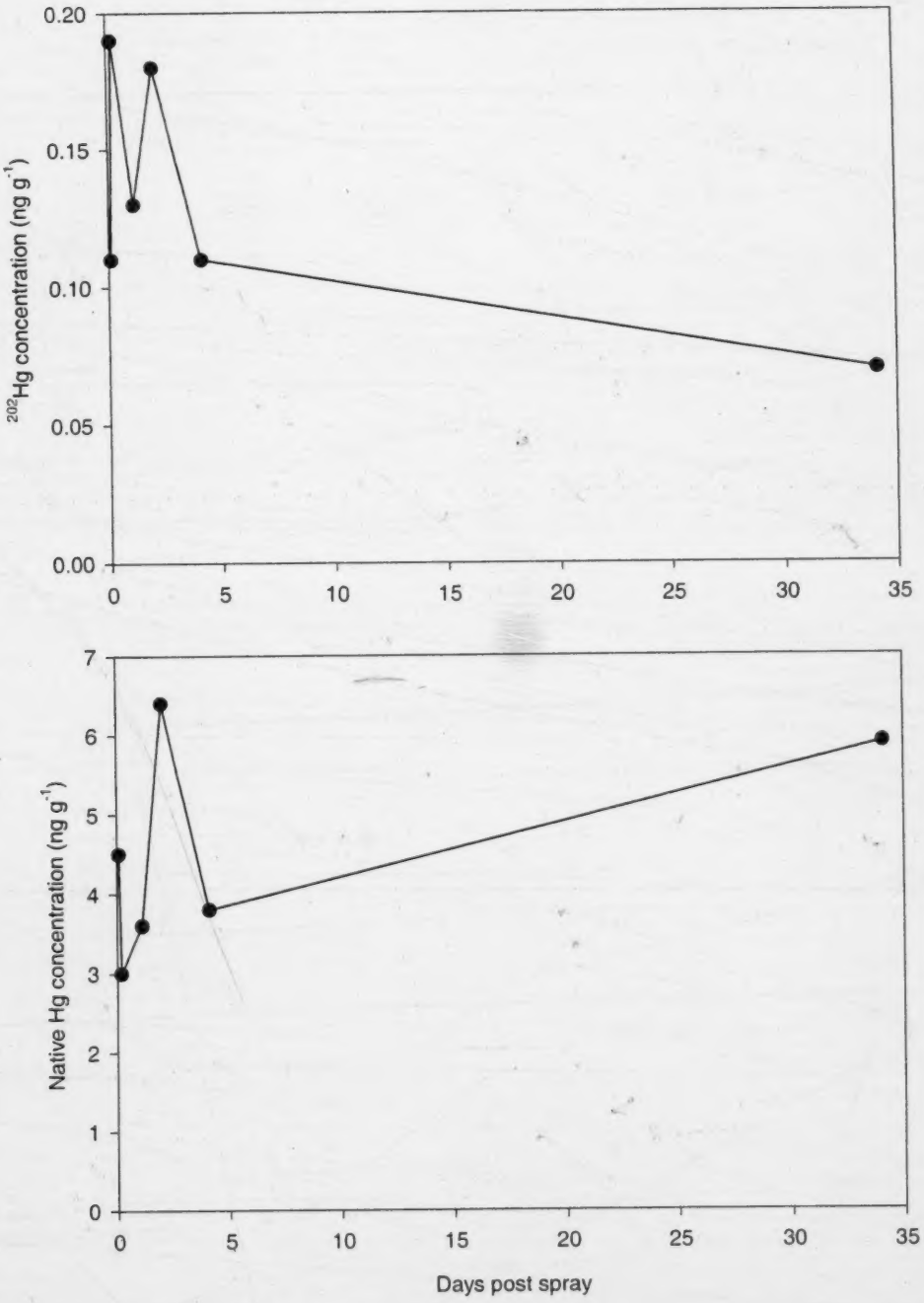

Figure 2.10 Concentration of ^{202}Hg (top panel) and native Hg (bottom panel) in jack pine foliage from 5 July 2001 spray-tree experiment over time.

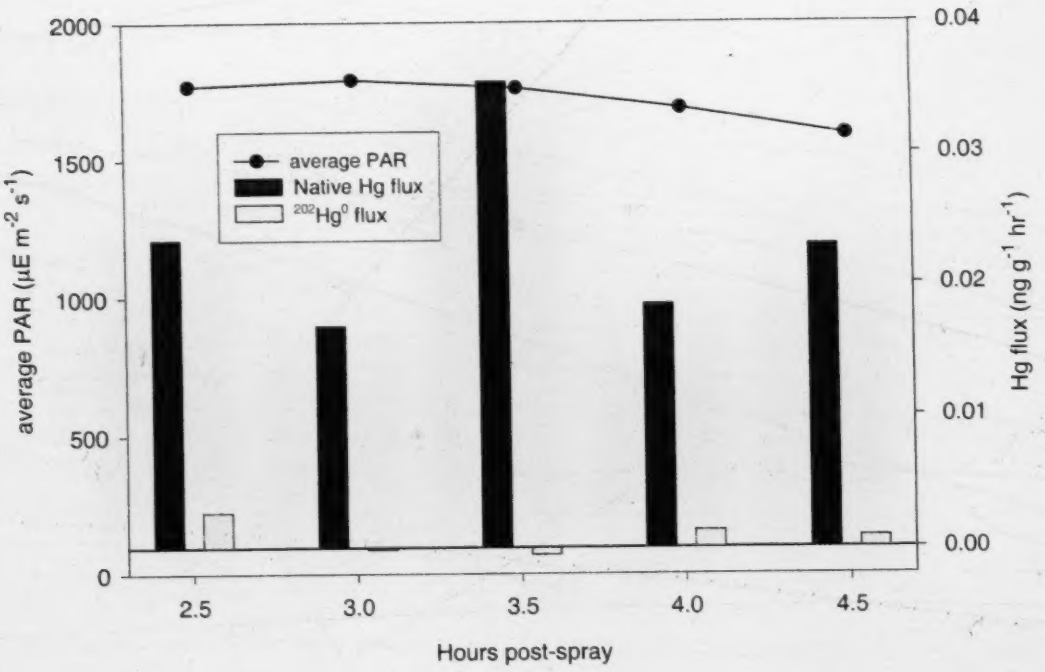

Figure 2.11 Ambient PAR conditions, $^{202}Hg(0)$ flux and native Hg(0) from black spruce foliage under different light treatments: Run 1 (2.5 hrs post-spray): full spectrum (no filter); Run 2 (3.0 hrs post-spray): PAR only treatment (using 226 Lee UV filter); Run 3 (3.5 hours post-spray): PAR+UV-A treatment (using Mylar type-D filter); Run 4 (4.0 hours post-spray): 2nd film layer control (using 2nd layer of Propafilm-C chamber wall material); Run 5 (4.5 hours post-spray): full spectrum (no filter).

In contrast to the native Hg(0) flux, ^{202}Hg(0) flux decreased over time in the absence of selective filters, from 2.68×10^{-3}, to 1.27×10^{-3}, to 0.85×10^{-3} ng g^{-1} hr^{-1} at 2.5, 4, and 4.5 and PAR + UV-A treatments both resulted in the flux of isotope becoming negative (i.e., isotope concentrations were higher in air entering the chamber than in air leaving it). Small concentrations of ^{202}Hg(0) were most likely present in air entering the chamber as a result of volatilization of photoreduced ^{202}Hg from the sprayed tree. Once UV filters were removed from the chamber, ^{202}Hg(0) again began to evade from foliage.

^{202}Hg concentration in black spruce foliage tended to decrease over time (Figure 2.12), but again an exponential loss was not observed. Native Hg concentrations stayed fairly constant or perhaps showed a slight increase over time. A large anomalous peak at 121 hours post spray was disregarded.

Again, rates of ^{202}Hg loss estimated from foliage concentrations were consistently higher than rates estimated using instantaneous flux measurements (e.g., 2.5×10^{-2} ng g^{-1} hr^{-1} based on foliage concentrations between 2.5-4.5 hours post-spray versus measured fluxes of 0.27×10^{-2} and 0.095×10^{-2} ng g^{-1} hr^{-1}, respectively).

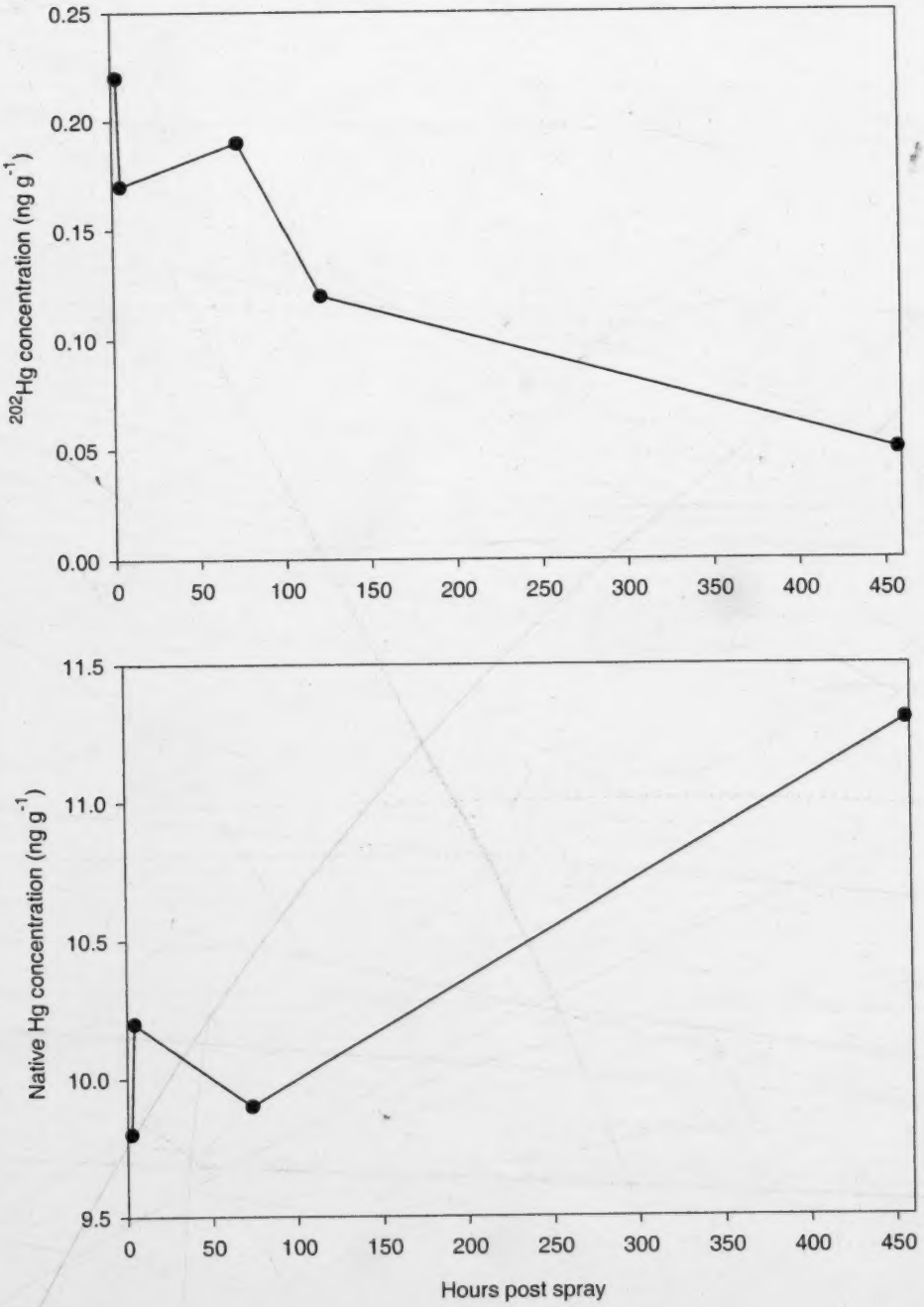

Figure 2.12 Concentration of ^{202}Hg (top panel) and native Hg (bottom panel) in black spruce foliage from 17 May 2002 spray-tree experiment over time.

Discussion

Based on observations that a significant proportion of ^{202}Hg(II) applied to jack pine and black spruce foliage in simulated precipitation was rapidly lost from the foliage post-application, we designed a dynamic chamber that interfered minimally with the physiological function of enclosed foliage to determine if ^{202}Hg(0) evasion rates could account for these losses. Results of experiments using selective UV filters suggests that presence of both PAR and UV wavelengths influence photochemical reduction of Hg(II) to Hg(0) and re-emission rates of newly deposited Hg(II) to foliage. However, measured fluxes of ^{202}Hg(0) could not account for the total observed reductions in tissue ^{202}Hg concentrations in either species, suggesting that processes other than photochemical reduction of Hg(II) contributed to the loss of Hg from foliage surfaces.

Effects of chamber on plant physiology (photosynthesis and transpiration)

Although irradiance levels in the laboratory were insufficient to cause light saturation points for photosynthesis, runs using the ADC chambers yielded APS rates for corn and loblolly pine that were consistent with published rates of maximum photosynthesis for corn and other C4 plants (20 - 40 μmol CO_2 m^{-2} s^{-1}, normalized to single leaf surface area) and slightly lower than those reported for temperate zone evergreen conifers (3 - 9 μmol CO_2 m^{-2} s^{-1}, normalized to optical projection of needles), respectively (Salisbury and Ross, 1992). The chamber designed to measure Hg(0) fluxes underestimated APS rates of corn compared to the smaller chamber (possible reasons for the discrepancy discussed below), and like the ADC chamber, its measurements of loblolly pine APS were slightly lower than the above reported range of values. Light saturation intensities for C3 plants are in the range of 400 -

500 μE m^{-2} s^{-1} (Hopkins, 1999), and this range is consistent with the leveling of loblolly pine APS rates at the highest light irradiances used in this experiment (350 - 450 μE m^{-2} s^{-1}). In contrast, APS rates of corn in both chambers showed no sign of saturation, consistent with the fact that C4 plants do not light saturate (Hopkins, 1999).

While the configuration of the Hg(0) flux chamber allowed normal physiological responses of the enclosed foliage, the flow rate was not sufficient to measure rapid changes in CO_2 assimilation. The lag period in detecting photosynthetic responses in the Hg(0) chamber was not surprising considering that the air in the chamber was only exchanged ~1 - 1.5 times per minute, whereas it was exchanged ~25 times per minute in the ADC broadleaf chamber. The conifer chamber had a similar air exchange rate to that of the Hg(0) flux chamber (1.5 exchanges per minute). The large size of the chamber used to measure Hg(0) flux also made it difficult to measure actual PAR intensities reaching the enclosed foliage. The portion of the corn leaf enclosed in the ADC broadleaf chamber received a uniform irradiance that was measured directly beside the chamber. Conversely, the large, folded leaf in the Hg chamber received different light intensities at different points along its length, resulting in a composite APS measurement of foliage photosynthesizing at different rates. Much closer CO_2 assimilation rates were observed between chambers using loblolly pine when this PAR measurement problem was addressed. Instead of securing the PAR sensor at one end of the chamber, measurements were taken on right and left sides, above and below, and in the center of the chamber at each light intensity. Because this gradient in PAR across the foliage would have been less important in field conditions under direct ambient sunlight, PAR measurements were simply taken from the sensor attached at a single location on the film wall of the chamber.

Losses of Hg from foliage following simulated precipitation events: comparison of losses measured using the flux chamber with decreases in foliage concentration over time

Year 2000

We observed exponential losses of ^{202}Hg from all foliage following application in simulated rainfall. This pattern of Hg loss is most readily explained by rapid reduction and volatilization of $^{202}Hg(II)$ in the spike solution. The ^{202}Hg remaining on foliage was likely the proportion that had become tightly bound to the leaf surface. A similar exponential loss of Hg(II) as Hg(0) has been observed in soils amended with Hg(II) salts (Hogg et al., 1978, Shlüter et al., 1995). These losses are inconsistent with a biotic reduction mechanism because there is no lag phase associated with enzyme induction (Grigal, 2002). Shlüter *et al.* (1995) hypothesized that reduction and volatilization of Hg slowed over time due to sorption of applied Hg to soil organic matter. Hg(II) has a strong affinity for the S-containing functional groups found in organic matter both in soils/sediments and in aqueous solution (Meili, 1991, Schuster, 1991, Kim *et al.*, 1997, Morel *et al.*, 1998, Shlüter, 2000). In aqueous solution, the Hg(II) reduction reaction involves an interaction between Hg(II) and dissolved organic ligands, specifically humic acids. Humic acids contain a free radical component of the quinine or semiquinone type that may function as an electron donor (Alberts et al., 1974). Therefore Hg(II)-humic acid complexes must be formed before the reduction of Hg(II) to Hg(0) can occur in aqueous solutions (Allard and Arsenie, 1991). The potential for transformation (and transport) of Hg(II) in soils therefore depends on the partitioning of organic matter between aqueous and solid phases (Schuster, 1991, Allard and Arsenie, 1991). While there are no published studies that examined the potential for Hg(II) reduction on

living foliar surfaces, we hypothesize that a similar interaction between Hg(II) and organic matter leads to volatilization of some proportion of newly deposited Hg(II) in rainfall.

The ability of living organisms to influence Hg(0) volatilization rates has been investigated for other taxa. For example, presence of algal (Ben-bassat and Mayer, 1975) and yeast (Brunker and Bott, 1974) cells in liquid culture enhanced production of Hg(0) when Hg(II) was added to the culture medium. In a field experiment at the ELA, volatilization rates of ^{202}Hg(0) from detached foliage placed on foil immediately after application of Hg(II) in simulated precipitation (96 ng m^{-2} hr^{-1} for birch and 131 ng m^{-2} hr^{-1} for jack pine) were much greater than from the foil surface sprayed with the same ^{202}Hg solution (9 ng m^{-2} hr^{-1}, all fluxes normalized to the areal footprint of the flux chamber) (S. Lindberg and V. St.Louis, unpublished data). It is unlikely that dissolved organic matter in the lake water used to mix the spike could have contributed to this enhanced reduction and evasion, since the same water was used in the foil application. It is therefore more likely that some component(s) of living foliar surfaces enhance(s) reduction and subsequent volatilization of Hg(II) compared to abiotic surfaces. Another possible source of reducing potential is organic material deposited to leaf surfaces in dry deposition (i.e., aerosols). This seems unlikely as well, since dry deposition of these aerosols is very low at the ELA (St.Louis *et al.*, 2001). It may be that components of leaf cuticles themselves are involved in forming reducible Hg(II)-organic complexes (resulting in the initial, rapid losses of spike as Hg(0)), and/or more stable, immobile Hg(II) complexes (resulting in the retention of Hg(II) and its slow loss over time). This bound fraction of applied Hg(II) may be lost by processes like leaf weathering and cuticle sloughing, but washing by rain appeared to have no effect on the initial rapid loss rates of spike, or on the subsequent slower decline in spike loss.

In the current study, the greater retention of ^{202}Hg over time on jack pine needles than on birch leaves may be due to differences in the reducing potential of their respective leaf surfaces, amount of light reaching the surfaces, and/or possibly differences in microclimate around the foliar surfaces. For example, temperature is known to affect Hg(0) volatilization rates via a direct effect on vapor pressure of Hg(0), and both biotic and abiotic reduction rates are also temperature dependent (Landa, 1978, Kim *et al.*, 1997, Grigal 2002). Possibly there was a lower retention of applied ^{202}Hg on birch leaves than jack pine needles because they were exposed to more light and wind action and subsequent weathering. It appeared that time of application (day vs. night) had no effect on the long-term retention of spike by either plant species, suggesting that ambient environmental conditions (e.g., possibly light or temperature) at time of Hg(II) deposition are not as important as those on several subsequent days in determining how much newly deposited Hg will remain on foliage.

Year 2001 & 2002

We observed an apparent interaction between $^{202}Hg(0)$ flux from jack pine needles and ambient light conditions in the 2001 spray tree experiment. When conditions were overcast on the day after spike application, there was no detectable flux of $^{202}Hg(0)$ from foliage. The following day was sunny, and fluxes of $^{202}Hg(0)$ were detectable once again. These results suggest that the instantaneous flux rate is influenced by ambient light conditions, and that the reduction of applied Hg(II) and subsequent volatilization of Hg(0) occurs on the leaf surface. The photochemical reduction of Hg(II) to Hg(0) is well documented for soils (Carpi and Lindberg, 1997, Zhang and Lindberg, 1999), water (Amyot *et al.*, 1994, Amyot *et al.*, 1997) and snow (Lalonde *et al.*, 2002). Increased rates of Hg(0)

production in the presence of Hg(II) and humic or fulvic acids in solution were observed under increased light intensities (Allard and Arsenie, 1991, Xiao *et al.*, 1995, Liu *et al.*, 2000). Factors affecting photochemical reduction included concentrations of reducible Hg(II)-humic acid complexes, and the intensity and wavelength of radiation (Morel *et al.*, 1998).

Interestingly, the flux of native Hg(0) from foliage surfaces in our study was not affected by ambient light conditions, and was detectable on the overcast day after spike application. This suggests a source of native Hg(0) other than the foliage surface, possibly from the transpiration stream through the stomata (Leonard *et al.*, 1998). However, the high levels of native Hg(0) evasion immediately post-spray may have been due to reduction of Hg(II)-humic acids in the lake water spray solution, or volatilization of dissolved gaseous Hg (DGM) also in the lake water.

The role of solar, and specifically UV, radiation in affecting Hg(0) fluxes was observed in the 2002 spray-tree experiment using black spruce. Omission of high-energy UV-A and UV-B wavelengths entering the chamber shut down the photochemical reduction of newly applied ^{202}Hg(II). Upon removal of selective filters, the evasion was restored, suggesting that intensity of ambient UV radiation influenced reduction of the newly deposited Hg(II) on foliage. UV wavelengths are extremely important in controlling formation of DGM in clear, high arctic lakes (Amyot *et al.*, 1997). In lakes with higher levels of dissolved organic carbon, UV was attenuated rapidly in the water column, and played a smaller role than visible radiation in influencing DGM production and volatilization (Amyot *et al.*, 1994, Amyot *et al.*, 1997). Similarly, in terrestrial areas that are well shaded and not exposed to direct sunlight (e.g., beneath a dense forest canopy), we hypothesize that the influence of both visible and UV light on Hg(0) fluxes would be smaller than in open, exposed areas (i.e., conditions of all spray tree experiments). Again, the magnitude and

direction of native Hg(0) flux was not affected by the removal of UV wavelengths, suggesting a non-surface, light-independent source of Hg(0).

The discrepancy between measured ^{202}Hg(0) fluxes in 2001 and 2002 experiments and corresponding ^{202}Hg loss rates determined from tissue concentrations indicates that either the chamber we designed to measure Hg(0) flux dramatically underestimates Hg(0) flux, or that there is another mechanism of Hg loss besides volatilization that remains unaccounted for. The flux chamber itself may have altered the microclimatic conditions surrounding the foliage so drastically that fluxes were underestimated by the order of magnitude observed. Intercomparisons of chambers measuring fluxes of Hg in naturally Hg-enriched areas led to the conclusion that chambers may suppress both diffusive and turbulent transport of Hg from surfaces, thereby underestimating the true flux (Gustin *et al.*, 1999a, Gustin *et al.*, 1999b, Wallschläger *et al.*, 1999, Lindberg *et al.*, 1999). Hg(0) fluxes estimated using chambers were ~3 times lower than those using micrometeorological techniques, which do not affect the microclimate under study (Gustin *et al.*, 1999b). Gustin *et al.* (1999b) concluded that suppression of fluxes by chambers occurred due to low air turnover rates that inadequately mimicked the turbulent flow conditions outside the chamber. In chambers without fans, the most important control over the boundary layer is the inlet flow rate. High flow rates help to decrease the concentration gradient between the air inside the chamber and the surface under study, hence minimizing suppression of the flux (Gustin *et al.*, 1999b, Gillis and Miller, 2000). The flux chamber in the present study had both an internal fan and a relatively high air flow rate, and therefore it seems unlikely that we grossly underestimated Hg(0) flux from the foliar surfaces. Weathering and cuticle sloughing has already been described as a possible loss mechanism of newly deposited Hg in rainfall and may account for the missing ^{202}Hg.

Conclusion

The tree spraying experiments indicate that a proportion of newly deposited Hg(II) in rainfall is likely reduced on the leaf surface via a reaction that may be influenced by ambient solar radiation and organic components of leaf surfaces. This Hg(0) is then volatilized to the atmosphere. The remaining Hg(II) is retained and slowly lost from the leaves over time, independently of washing by rainfall. The discrepancy between loss rates of newly deposited ^{202}Hg(II) as determined from tissue concentrations, and measured ^{202}Hg(0) fluxes indicates that there may be mechanisms of loss of Hg(II) other than photochemical reduction to Hg(0). Possible explanations include weathering or cuticle sloughing. The other possibility is that reduction and evasion is the only mechanism at work, and the chamber used in this study simply underestimated Hg(0) flux due to interference with leaf surface microclimatic conditions. This seems unlikely considering the design of the chamber and its high flow rate. Further studies are needed to accurately estimate losses of Hg(II) from foliage via other possible pathways, and further chamber intercomparison studies should illucidate the effects of chamber configuration on the measured flux.

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Chapter 3 Mechanisms of Hg accumulation in foliage: evaluation of three possible input pathways using stable Hg isotopes

Introduction

The dropping of senescent foliage in litterfall represents the largest flux of mercury (Hg) to many terrestrial forested landscapes (Driscoll *et al.*, 1994, Lindberg, 1996, St.Louis *et al.* 2001). Due to the magnitude of this flux, identifying the source(s) of Hg in litterfall is imperative for a correct understanding of the biogeochemical cycling of Hg in forests (Lindberg, 1996). Potential sources of Hg in foliage include root uptake of soil-water Hg via roots and subsequent translocation to foliage, retention of Hg(II) deposited in rain events, and stomatal uptake of gaseous elemental Hg (Hg(0)). There is evidence from laboratory studies that root uptake of Hg from soil does not contribute significantly to Hg content of leaves. Roots act as a significant site for Hg binding, and little translocation of Hg to foliage occurs (Beauford *et al.*, 1977, Lindberg *et al.*, 1979, Godbold and Hüttermann, 1988). Observations that Hg content of throughfall (precipitation that passes through the canopy) is higher than that of open area precipitation suggests that forest canopies do not likely scavenge and retain Hg(II) from precipitation, but instead contribute to this flux of Hg to the forest floor (St.Louis *et al.*, 2001). In contrast, a number of field observations indicate that stomatal uptake of atmospheric Hg(0) may account for the bulk of Hg in leaves and litterfall. For example, foliar Hg concentrations are often positively related to the length of growing season (Fleck *et al.*, 1999), and Hg concentrations of leaves increases with age of the tissues (Bombosch, 1983, Barghigiani *et al.*, 1991, Rasmussen *et al.*, 1991, Wyttenbach and Tobler, 1988). Although there is evidence to support these general conclusions, it is difficult to quantify relative amounts of Hg from these two pathways since Hg derived from different sources is indistinguishable using conventional Hg measurement techniques.

The goal of this study was to *directly* examine the processes of root uptake of soil Hg, retention of wet deposition Hg(II), and stomatal uptake of atmospheric Hg(0) using stable Hg isotopes as tracers. Use of stable Hg isotopes allows various uptake pathways to be evaluated independently depending on how the Hg isotope is introduced to the plant. In this study, stomatal uptake was examined using a gaseous $^{198}Hg(0)$ in conjunction with a dynamic flux chamber for measuring Hg(0) exchange from canopy foliage. It was hypothesized that if stomatal uptake of Hg occurred, there would be less ^{198}Hg leaving the chamber containing foliage than than entering, and the ^{198}Hg would be detectable in foliage itself. Root uptake of Hg and translocation to foliage was investigated using a short term ^{202}Hg dose-response experiment and a three year microcatchment-scale study that utilized multiple Hg isotopes applied to ground vegetation. We hypothesized that if root uptake was indeed an insignificant pathway of Hg accumulation in foliage, the ground-applied isotopes should not be present in canopy foliage. In addition, Hg(0) fluxes from the foliage of the exposed plants (measured using the dynamic flux chamber) should not contain soil-applied isotopes at detectable levels. Retention of Hg(II) from simulated precipitation was monitored by following concentrations of the applied isotopes in ground vegetation over time.

Methods

Deposition of atmospheric Hg(0) to foliage: ^{198}Hg(0) permeation tube experiment

The Hg(0) flux chamber was used in conjunction with a ^{198}Hg(0) tracer to examine uptake of atmospheric Hg(0) by foliage. The goal of these experiments was to replace the native Hg(0) present in air entering the chamber with approximately equal amounts of ^{198}Hg(0). It was hypothesized that if direct deposition of Hg(0) to foliage occurred, it would be possible to detect the isotope in/on the plant tissues. It was also hypothesized that concentrations of ^{198}Hg(0) entering the chamber would be higher than concentrations leaving if foliar uptake of Hg(0) occurred.

^{198}Hg(0) was introduced into the chamber using a custom-made permeation tube (Vici metronics Inc., Santa Clara, CA). The permeation tube was a sealed Teflon tube with an active length of 3.7 cm that contained 6.4 mg of 90.5 % enriched liquid ^{198}Hg(0). The tube was placed in a glass U-tube immersed in a 30°C water bath to maintain a constant rate of ^{198}Hg(0) release from the tube. A pump regulated by a mass flow controller delivered 1 L min^{-1} of air scrubbed through a gold trap through the U-tube and into the main chamber inlet line. Under these conditions, the permeation tube released 0.022 ng ^{198}Hg(0) min^{-1} into the main line. The main line also contained a large gold trap downstream of the pumps to prevent the majority of native Hg(0) from entering the chamber. The total air input rate to the chamber (main line plus the permeation tube line) was maintained at around 8.6 - 8.9 L min^{-1} for all experiments. To quantify concentrations of ^{198}Hg(0) and native Hg(0) entering and leaving the chamber, the inlet and outlet lines were continuously sub-sampled through gold traps. Over the course of each experiment, gold traps were replaced every hour to prevent saturation of Hg(0) binding sites. The actual average inlet air ^{198}Hg(0) concentration

(± standard error) for all runs was 1.24 (± 0.03) ng m^{-3}. Concentration (± standard error) of native Hg(0) entering the chamber was 1.69 (± 0.16) ng m^{-3}, as it proved to be extremely difficult to remove all native Hg(0) entering the chamber at high flow rates. On average we delivered a total of 2.9 ng m^{-3} of Hg(0) to the chamber.

A birch (*Betula papyrifera*) tree and a jack pine (*Pinus banksiana*) tree were selected in an open, ridgetop area at the Experimental Lakes Area (ELA) in northwestern Ontario. Because concentrations of Hg in foliage appear to increase with tissue growth (Rasmussen, 1995), we conducted our experiments early in the growing season. The first experimental run was carried out on the birch tree on 30 May 2002. At this time, small leaves approximately 1 cm across had emerged from buds. A second run was done on 6 June 2002 when leaves had grown to approximately 4 cm across. The final run was performed on the jack pine tree on 20 June 2002 at which point new needle growth was very prominent.

Branches were placed into the chamber and allowed to equilibrate at full flushing rates for about 15 minutes prior to introduction of $^{198}Hg(0)$. Sampling of inlet and outlet air streams commenced as soon as the pump on the $^{198}Hg(0)$ spike input line was turned on. For the few moments it took to change the inlet and outlet gold traps every hour, the pump delivering the isotope was switched off to ensure accurate quantification of the total spike applied. We introduced $^{198}Hg(0)$ for three hours to the birch tree (each run), and for six hours to the jack pine. Following exposure, the enclosed branch was collected using clean sampling techniques. Birch leaves were separated from branches, and new jack pine needles were separated from year-old ones. Tissues were freeze-dried, weighed, and homogenized using clean stainless steel coffee grinders.

100 mg sub-samples of the homogenized tissues were digested in 20 mL glass vials using 10 mL of a 7:3 (vol:vol) mixture of concentrated nitric and sulfuric acids. Vials were

covered with glass marbles and samples were heated on a hot plate at 80 °C under slow reflux until the production of brown nitrous gases ceased. Samples were diluted with water as needed. THg content of the digests was determined by continuous flow injection cold vapor generation with ICP/MS detection (Finnigan MAT, Model Element 2). The acidified samples were continuously mixed with a solution of stannous chloride (reductant) using peristaltic pumps. The mercury vapor formed was separated in a gas liquid separator (Model LI-2) and the Hg(0) swept into the plasma of the ICP/MS (Hintelmann and Ogrinc, 2002, in press). Limits of detection (LOD) for Hg isotopes depended on the concentration of native Hg present in the sample. Using the techniques described above, to detect an applied isotope with certainty, it had to have a concentration >0.5-1% of native Hg. The LOD varied with the precision of the isotope ratio measurement during each run, and for these samples ranged from 0.2-1 ng g^{-1} (Hintelmann *et al.*, 2002). Gold traps were analyzed by thermal desorption into a carrier gas and detection also involved ICP/MS.

Uptake of soil Hg by plants: uptake of isotope applied to soils under trees

The soils beneath 12 small pin cherry (*Prunus pensylvanica*), 12 small jack pine, and 12 small birch trees were dosed with either 0 μg, 4 μg, 8 μg, 20 μg of ^{202}Hg (three replicates of each load). Prior to isotope application, 1 m^2 wooden frames were constructed around the base of each tree (Figure 3.1). A plastic film was stapled to the frame and duct taped around the base of the tree. These frames were elevated approximately 15 cm above the ground to allow air to pass below the plastic, but prevent the direct upward movement of any $^{202}Hg(0)$ volatilized soon after loading. These plastic covers remained in place for four days. Soils under the pin cherry and jack pine trees were dosed on 3 May 2001, whereas soils under

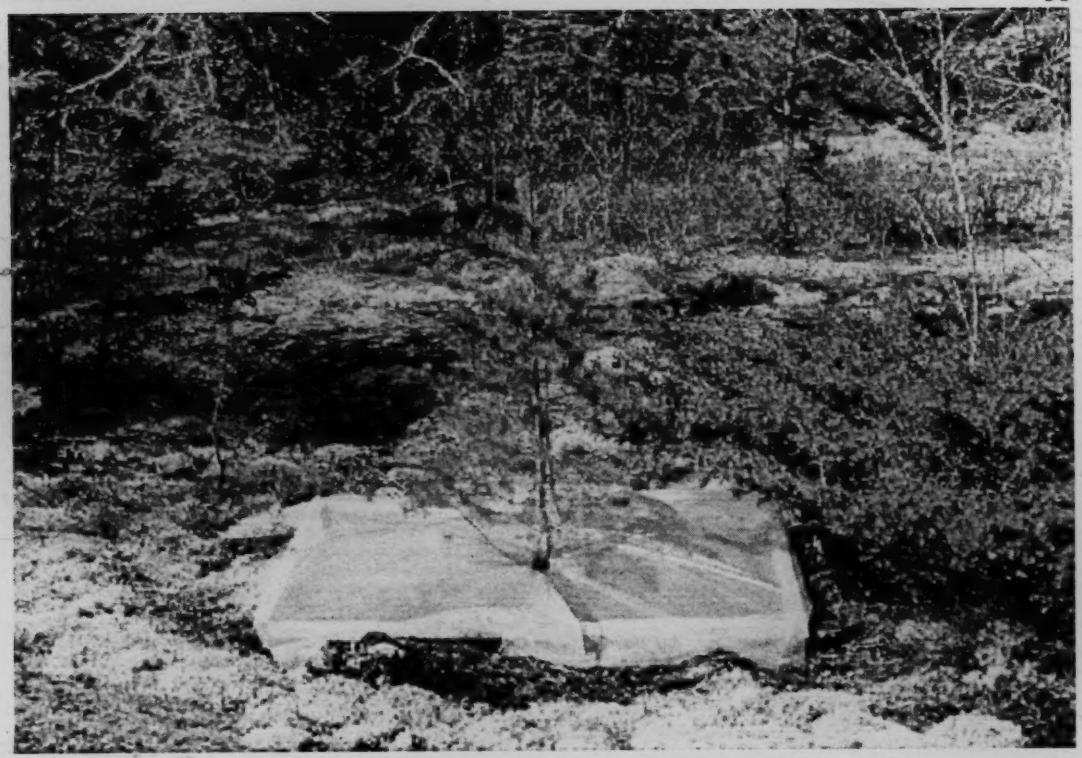

Figure 3.1 Plastic film and wooden frames beneath small jack pine to prevent direct deposition of volatilized, ground-applied $^{202}Hg(0)$ to foliage (photo provided by Vince St.Louis).

birch trees were dosed on 13 May 2001. Spike solution was made for each tree by adding inorganic divalent ^{202}Hg (99.2% enriched) and NaOH to 1 L of Winnange Lake surface water (Table 3.1). Spikes were mixed in a plastic garden sprayer, and treatments were applied in order of increasing concentration.

Leaves from the pin cherry and birch trees, and needles from jack pines, were sampled weekly in the month following spike application, and monthly after that. 25 July 2001 samples were selected for preliminary isotope analysis. For the jack pine samples, year-old foliage was separated from new growth and analyzed separately. Samples were dried, homogenized, and analyzed for native and ^{202}Hg as described above.

Uptake of soil Hg by plants: retention and uptake of isotopes applied to the ground vegetation in a forested micro-catchment

U1F isotope applications

The U1F micro-catchment is a well-characterized 680 m^2 plot in the uplands of the Lake 302 watershed (Figure 3.2). Approximately 642 m^2 of the plot is densely forested with mature jack pine and black spruce trees. The remaining area is open, of which 7.4 m^2, 5.8 m^2, and 24.7 m^2 is covered primarily by lichen, moss (*Pleurozium* sp.) and a mixture of blueberry (*Vaccinium* sp.), moss and lichen, respectively. Inorganic Hg loading to this area was initially increased experimentally in the summer of 1999. On 13 July 1999, 7.4 mg of inorganic divalent ^{202}Hg (99.2% enriched) was applied evenly onto the ground vegetation of the micro-catchment. The isotope stock solution was made by dissolving 20 mg of $^{202}Hg(0)$ in 500 mL of dilute HCl. Twenty-one mL of this solution was added to each of 10 carboys containing 18.5 L of throughfall collected from under a jack pine and black spruce canopy in a nearby microcatchment. The pH was adjusted to 5.0 by adding 40 mL of 0.68 M NaOH, and the

Table 3.1 Amounts of ^{202}Hg primary spike solution and NaOH in 1 L Winnage Lake water applied to soil beneath pin cherry, jack pine, and birch trees at four different treatment levels.

Total mass ^{202}Hg added to plot (μg)	Volume (μL) of 40 mg L^{-1} ^{202}Hg spike solution	Volume (μL) of 27% NaOH
0	0	0
4	100	20
8	200	40
20	500	100

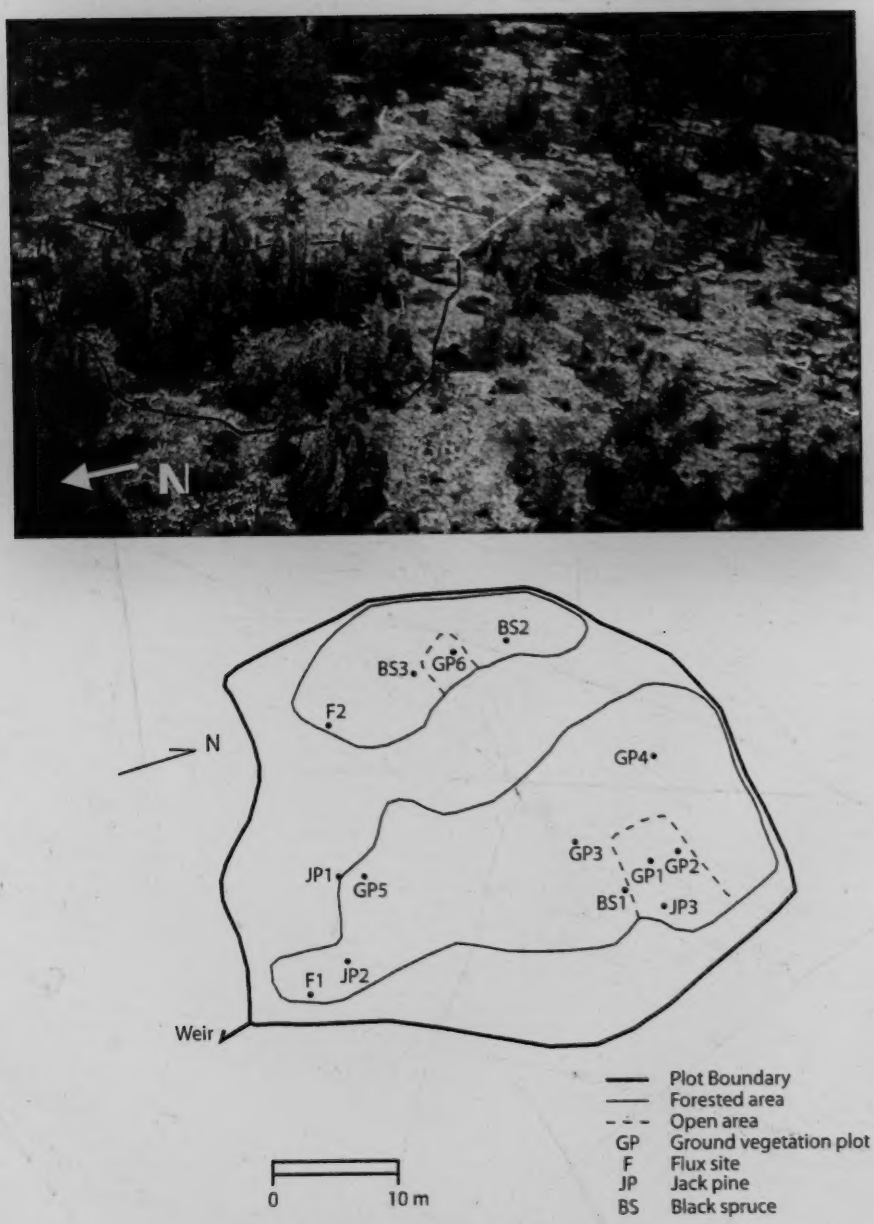

Figure 3.2 Aerial photograph (top panel, photo by John Shearer) and map (bottom panel) of U1f microcatchment (modified after Allan *et al.*, 1993) showing flux measurement sites for jack pine (F1) and black spruce (F2) trees, ground vegetation sampling sites, and canopy vegetation sampling sites (JP & BS).

solution was allowed to equilibrate for 4 hours prior to application. The microcatchment was divided into 45 15 m^2 sections, and 4.1 L of the spike solution was applied to each subplot using plastic garden sprayers. In addition to the vegetated areas, a 33 m^2 strip of bedrock connecting the two forested areas within the plot was also sprayed. Based on measurements from the sprayers, the concentration of the applied spike was 39.8 ± 0.9 $\mu g\ L^{-1}$. The spike was applied in the late afternoon to minimize volatilization, which may be enhanced by exposure to direct sunlight. This application technique simulated a small rain event or dry deposition rather than a large precipitation event. This single application elevated the annual wet deposition of total Hg in the open to the area (7 $\mu g\ m^{-2}\ y^{-1}$, St. Louis *et al.*, 2001) by 10.9 $\mu g\ m^{-2}$, to levels comparable to those observed in urban areas of the United States (Hintelmann *et al.*, 2002). In 2000, ^{200}Hg was applied in a similar fashion to the micro-catchment. However, instead of applying the spike all at once, it was applied monthly beginning in May and ending in September in applications of 2.5 $\mu g\ m^{-2}$. In 2001, ^{201}Hg was applied in an identical fashion as in 2000 with monthly applications began in May.

Canopy vegetation

Three jack pine and three black spruce trees in the U1F micro-catchment were sampled to determine if canopy foliage contained detectable levels of any Hg isotopes applied to ground vegetation (Figure 3.2). Foliage was collected using a pole-pruner and ultra-clean sampling techniques from approximately 3 m (high foliage) and 1 m (low foliage) heights above ground. Sampling occurred on the following dates: 10 Sept 1999, 5 May 2000, 9 June 2000, 28 Sept 2000, and 25 July 2001. For the first 4 sampling dates, a composite sample was homogenized and analyzed without differentiating needles from branches, or ages of tissues. Needles from the 25 July 2001 samples were separated into new and year-old

growth, and for each age, needles were separated from branches to examine patterns of Hg accumulation in different tissues. All U1F plant tissues were freeze-dried and then homogenized using clean stainless steel coffee grinders. Digestion and analysis was the same as described for the permeation tube experiment tissues.

Ambient and isotopic fluxes from black spruce and jack pine in U1F

It was hypothesized that if root uptake and translocation of the ground-applied isotopes was occurring, Hg fluxes from canopy foliage within the catchment might contain a detectable isotopic signal. To examine this possibility, the dynamic Hg flux chamber described previously was used to measure isotopic and native Hg(0) fluxes from a small jack pine and a small black spruce within the micro-catchment on 24-25 May 2001 (Figure 3.2). Flux measurements lasted 30 minutes, and were repeated on two separate branches from each tree. Flux branches were collected and analyzed for total leaf area using area measurement software (SigmaScan, Jandel Scientific)..

Ground vegetation

Six sites in both open and forested areas of the U1F micro-catchment containing different vegetation types were selected in 1999 to follow accumulation/loss of the applied isotopes over time (Figure 3.2). The estimated total ground area covered by the vegetation types in the representative sites is in parentheses: 1) lichen (7.4 m^2); 2) moss (*Pleurozium* sp.)/lichen/blueberry (*Vaccinium* sp) (30.5 m^2); 3) *Pleurozium* sp. (161 m^2); 4) *Pleurozium* sp./*Vaccinium* sp. (161 m^2); 5) primarily *Vaccinium* sp. (161 m^2); and 6) primarily *Vaccinium* sp. with some *Pleurozium* sp. (161 m^2). Ground vegetation within 625 cm^2 quadrats was collected by carefully removing the upper layer of living plant material from the underlying soil layer. Attempts were made to exclude as much soil and leaf litter from the samples as possible. The aboveground portions of shrubs were sampled using clean hand-pruners. Lichens and

mosses were peeled off the soil/rock using gloved hands. Material from the plots was stored frozen in Ziploc bags, and after freeze drying, total dry weight of vegetation from each plot was measured. Following analysis as described above for canopy foliage, native and isotopic concentrations of Hg were multiplied by areal vegetation biomass (g m^{-2}) to estimate areal mass of Hg (μg m^{-2}) in the aboveground vegetation. To obtain a total mass of ambient and isotopic Hg in ground vegetation in the U1F micro-catchment, the areal mass measured in the plots was then multiplied by the estimated total area of the community in U1F.

Results

Deposition of atmospheric Hg(0) to foliage: ^{198}Hg(0) permeation tube experiment

There appeared to be a deposition of ^{198}Hg(0) to foliage using the difference in concentrations of ^{198}Hg(0) entering and leaving the chamber (Figure 3.3). The rate of spike deposition to birch leaves increased from near zero (0.062 ± 0.068 ng g^{-1} hr^{-1}) when leaves were very small, to 0.410 ± 0.045 ng g^{-1} hr^{-1} when leaves were larger. There also appeared to be a small deposition of ^{198}Hg(0) to jack pine foliage (0.039 ± 0.002 ng g^{-1} hr^{-1}).

Concentrations of ^{198}Hg in the foliage within the chamber during runs were all below the 1% of native Hg LOD, except in run 2 birch leaves (0.022 ng g^{-1}; 1.07% of native Hg) (Table 3.2). However, all leaf tissue samples did show excess ^{198}Hg content in relation to expected natural abundance levels (Table 3.2). In contrast, no excess ^{198}Hg was detected in any stem samples.

In all three runs, the measured tissue concentrations of ^{198}Hg were too low to account for the estimated deposition of ^{198}Hg(0) using concentrations entering and leaving

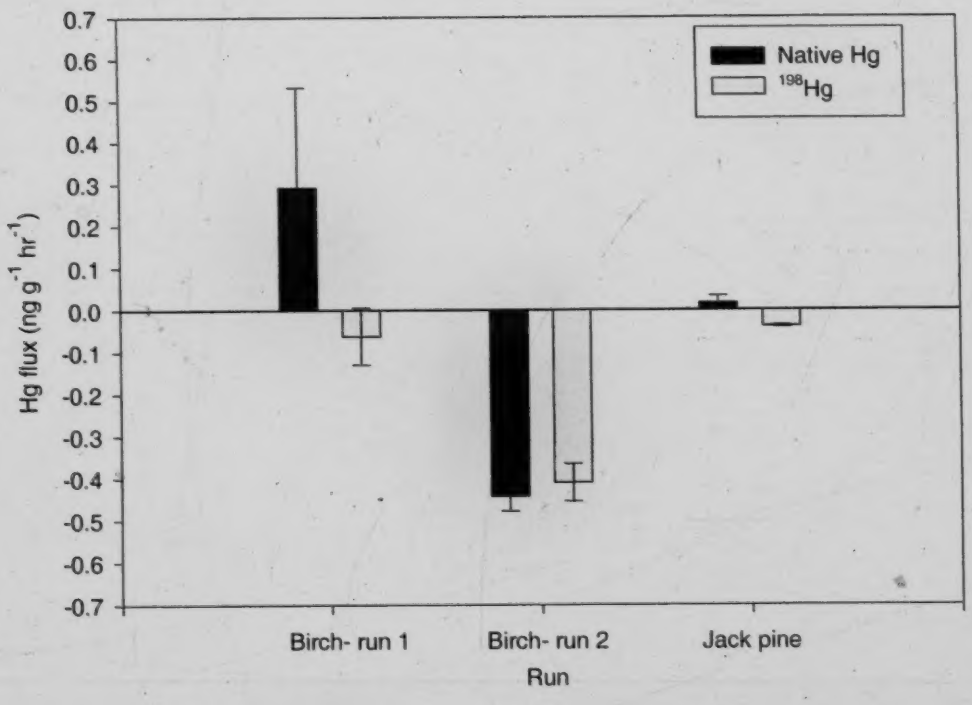

Figure 3.3 Exchange of $^{198}Hg(0)$ and native Hg(0) from birch and jack pine foliage in three permeation tube experiment runs (May-June 2002)

Table 3.2 Native Hg and ^{198}Hg concentrations of dissected birch and jack pine tissues from three $^{198}Hg(0)$ permeation tube experiment runs. Concentrations below the 1% of native Hg LOD are highlighted. Standard reference material is NIST apple leaves.

Run	Tissue type	Native Hg concentration ($ng\ g^{-1}$)	^{198}Hg concentration ($ng\ g^{-1}$)	% ^{198}Hg of Native Hg
Young birch (May 30, 2002)	Current-year leaves	1.7	0.009	0.53
	Stems	9.0	ND	-
Older birch (June 6, 2002)	Current-year leaves	2.0	0.022	1.07
	Stems	7.3	ND	-
Jack pine (June 20, 2002)	Current year (needles + stems)	2.7	0.004	0.14
	Previous years' needles	7.7	0.016	0.21
	Previous years' stems	14.7	ND	-
NIST SRM	Apple leaves	43.2	0.008	0.02

the chamber. For example, in the first birch run, the estimated deposition rate should have resulted in 0.04 ng ^{198}Hg in tissues. In fact, only 0.0018 ng ^{198}Hg could be accounted for by measuring tissue concentrations, and this number is highly uncertain, as concentration of the isotope in this sample was below the LOD (0.53% of native Hg). The discrepancy is worse for the second birch run, where total estimated ^{198}Hg(0) deposition using the chamber was 0.73 ng. In contrast, only 0.013 ng could be accounted for in leaves. Even if the remainder of the missing spike was present in stems, it should have been detectable as it would have been equal to 1% of native Hg concentration. Jack pine tissue concentrations are also approximately an order of magnitude lower than expected based on estimated deposition rates.

Direction of native Hg(0) flux did not necessarily always correspond to direction of ^{198}Hg(0) flux in these three runs. In the first birch and jack pine runs, native Hg flux from foliage was positive (evasion) while in the second birch run, the flux was negative (deposition) (Figure 3.3). Native Hg concentrations were higher in older tissues than in new growth (Table 3.2). For example, in the birch trees, concentrations of native Hg in stems were on average 4.5 times higher than in new leaves, and in jack pines, year-old needles had almost 2.9 times as much native Hg as new growth.

Uptake of soil Hg by plants: uptake of isotope applied to soils under trees

No excess ^{202}Hg was detected in any of the 25 July 2001 samples. Root uptake and translocation to leaves or direct deposition (via volatilization) of ^{202}Hg applied to soils did not occur to any detectable extent over the short 2.5 month period between soil dosing and foliage collection. Average native Hg concentrations of new jack pine, birch, and pin cherry

foliage were similar and ranged from 5.2 ± 0.5 ng g^{-1} in birch to 6.6 ± 0.4 ng g^{-1} in pin cherry (Figure 3.4). Average concentrations of native Hg in year old jack pine needles (10.3 ± 0.7 ng g^{-1}) were almost twice that in new growth (5.7 ± 1.8 ng g^{-1}).

Uptake of soil Hg by plants: retention and uptake of isotopes applied to the ground vegetation in a forested micro-catchment

Canopy vegetation

The foliage of black spruce and jack pine trees growing in the U1f microcatchment had no detectable concentration of excess ground-applied ^{202}Hg, ^{200}Hg or ^{201}Hg when collected on 25 July 2001. Both species had similar native Hg concentrations in high (jack pine 10.8 – 16.5 ng g^{-1}; black spruce 10.6 – 16.8 ng g^{-1}) and low foliage (jack pine 12.6 – 20.3 ng g^{-1}; black spruce 14.8– 25.2 ng g 1). High foliage samples had significantly lower native Hg concentrations than low foliage samples for both jack pine (repeated measures ANOVA, failed normality test, p=0.004, α=0.05, n=3) and black spruce (p=0.02, α=0.05, n=3) (Figure 3.5). Native Hg content of year-old needles was significantly higher than current-year needles for jack pine (3.34 vs. 8.23 ng g^{-1}; paired t-test, p=0.024, α=0.05, n=3) and black spruce trees (4.47 vs. 7.60 ng g^{-1}; paired t-test, p=0.003, α=0.05, n=3) (Figure 3.6).

Ambient and isotopic fluxes from black spruce and jack pine in U1F

No detectable concentrations of any soil-applied isotopes were found in air leaving the flux chamber (Figure 3.7). In 50% of runs, trace amounts of excess ^{200}Hg and/or ^{202}Hg were detected in air entering the chamber (most likely derived from the intake line picking

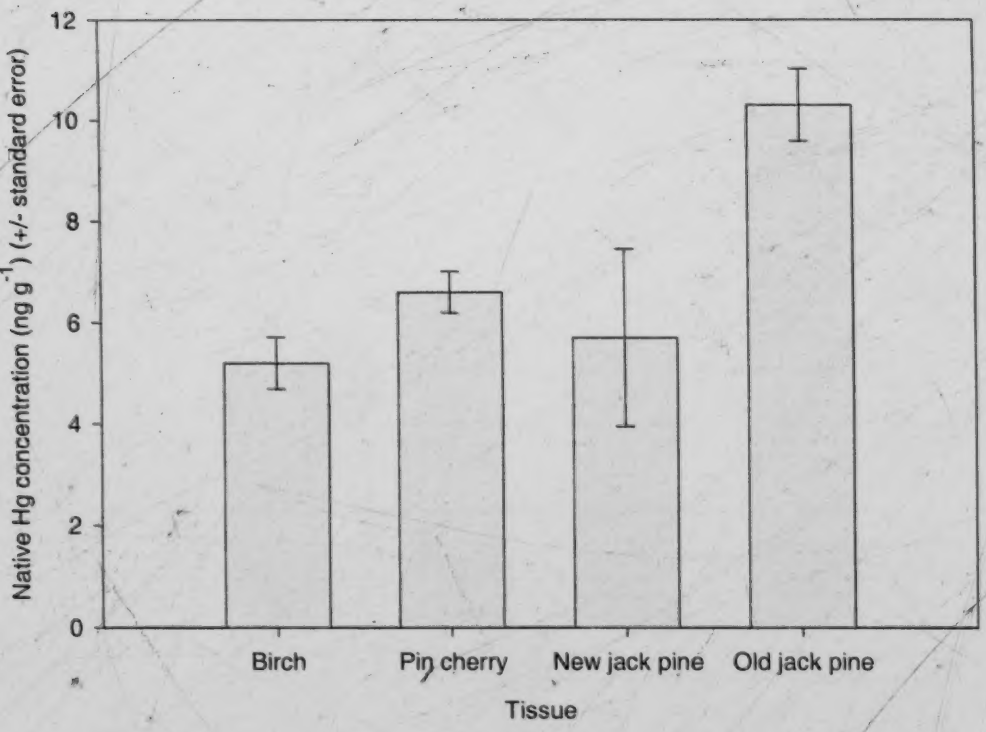

Figure 3.4 Average native Hg concentration of current-year (2001) birch (n=13), pin cherry (n=11), and jack pine trees (n=12) and year old (2000) foliage of jack pine trees (n=12) treated with ground applied ^{202}Hg at 4 different treatment levels.

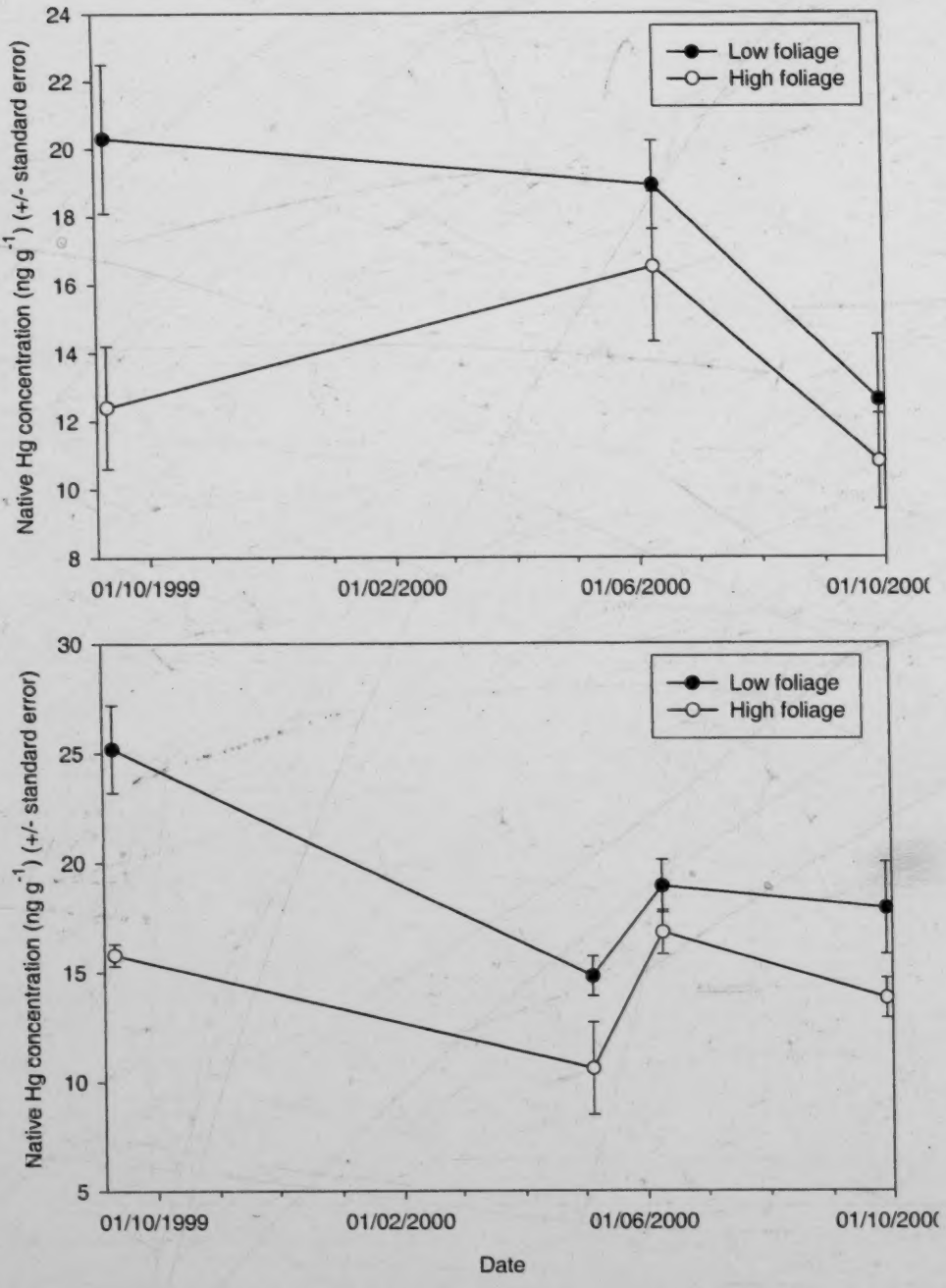

Figure 3.5 Average native Hg concentration of high and low foliage of U1f jack pine (top panel, n=3) and black spruce (bottom panel n=3) trees.

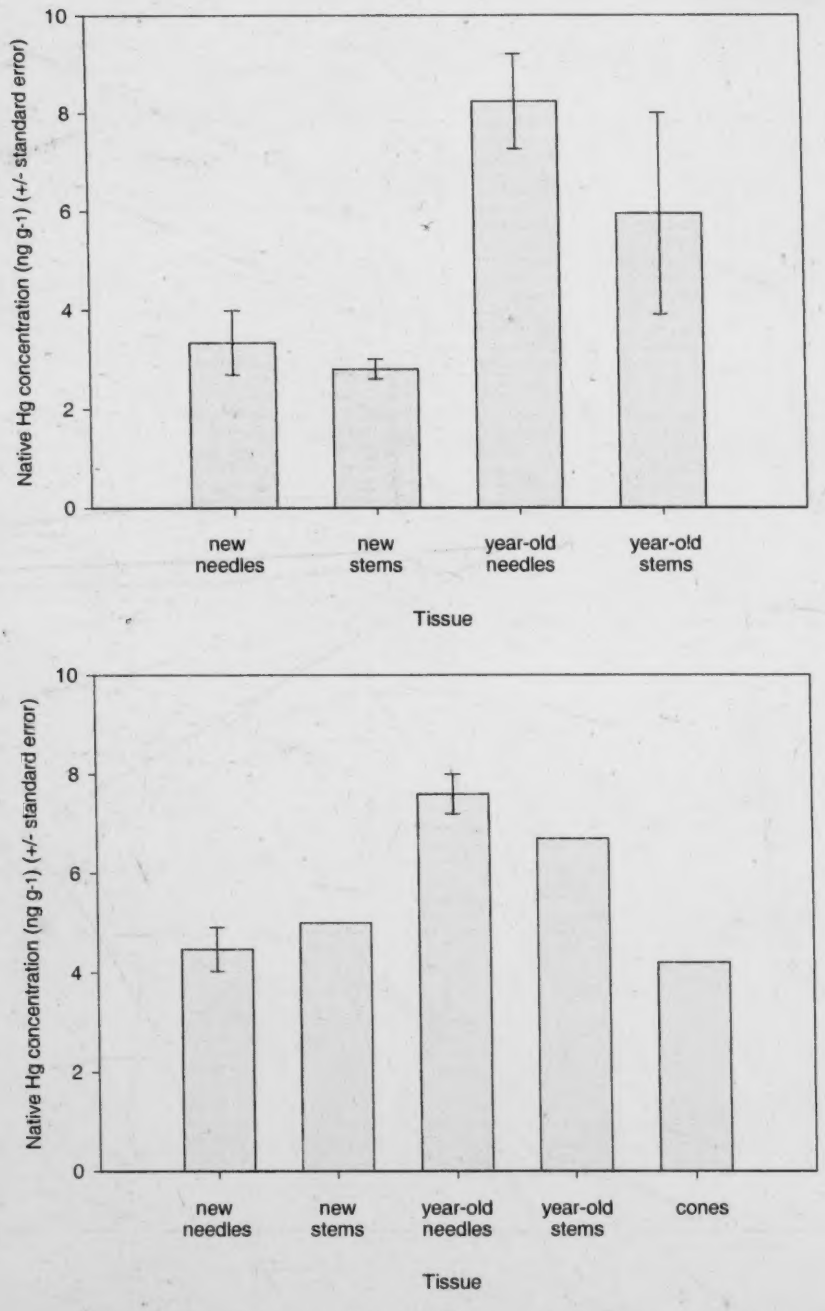

Figure 3.6 Average native Hg concentrations of dissected U1f jack pine (top panel) and black spruce (bottom panel). All tissues without error bars represent single samples, and for those with error bars n=3.

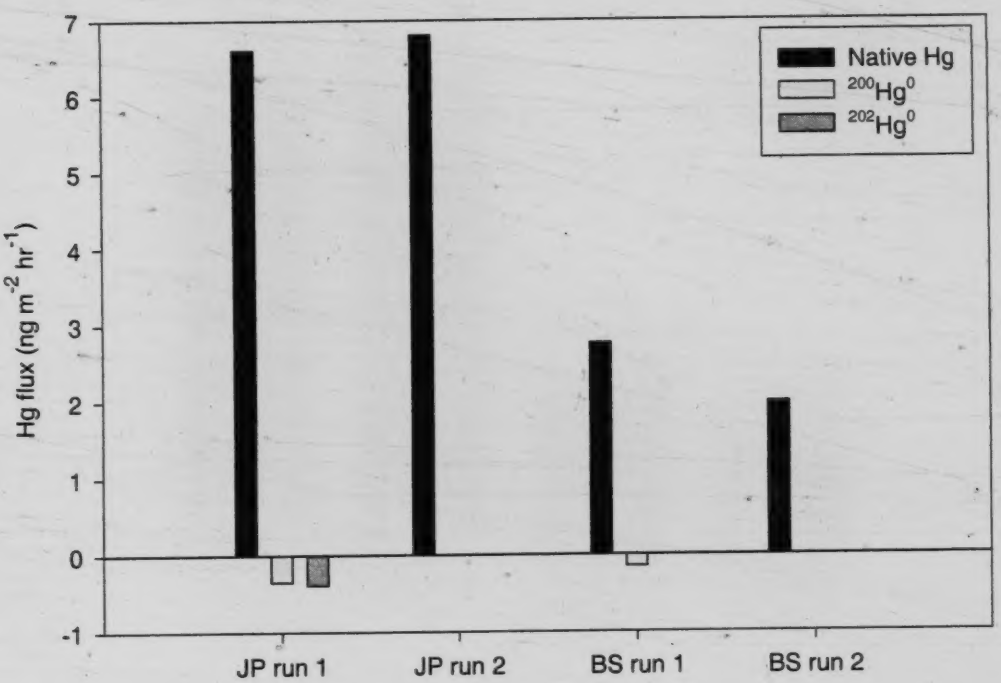

Figure 3.7 Native Hg(0), $^{200}Hg(0)$, and $^{202}Hg(0)$ fluxes from U1f jack pine (JP, May 24, 2001) and black spruce (BS, May 25, 2001) trees.

up Hg isotope being volatilized from the ground vegetation where it was applied), indicating the possibility of deposition of Hg(0) to canopy foliage within the canopy (Figure 3.7).

Ground vegetation

Ground vegetation quadrats had varying concentrations of Native Hg depending on the plant species present. Native Hg concentrations in the mostly blueberry quadrat (5) ranged from 13.0 - 63.0 ng g^{-1}, whereas quadrats containing mostly lichen (1) or moss (3) contained higher concentrations ranging from 46.7 - 56.6 ng g^{-1} and 79.7 - 118.8 ng g^{-1}, respectively. Quadrats containing a mixture of these vegetation types (2, 4, & 5) had a wide range of concentrations, reflecting their varied composition (41.2 - 163.3 ng g^{-1}).

Areal biomass and mass of native Hg appear to follow a seasonal pattern, increasing over the growing season, and decreasing in the fall (Figure 3.8). In contrast, areal mass of the excess ^{202}Hg applied once in 1999 showed a gradual decrease from estimated initial application levels (10.90 μg m^{-2}) over time to a minimun of 2.38 ± 0.79 μg m^{-2} on 30 August 2001 (Figure 3.8). Areal mass of the excess ^{200}Hg applied in 2000 increased gradually with monthly applications and leveled-off around 7μg m^{-2}. Similarly, the areal mass of the excess ^{201}Hg applied monthly in 2001 showed an initial increase from 0 before the first spike application in May 2001 to 3.20 ± 0.53 μg m^{-2} by 30 August 2001.

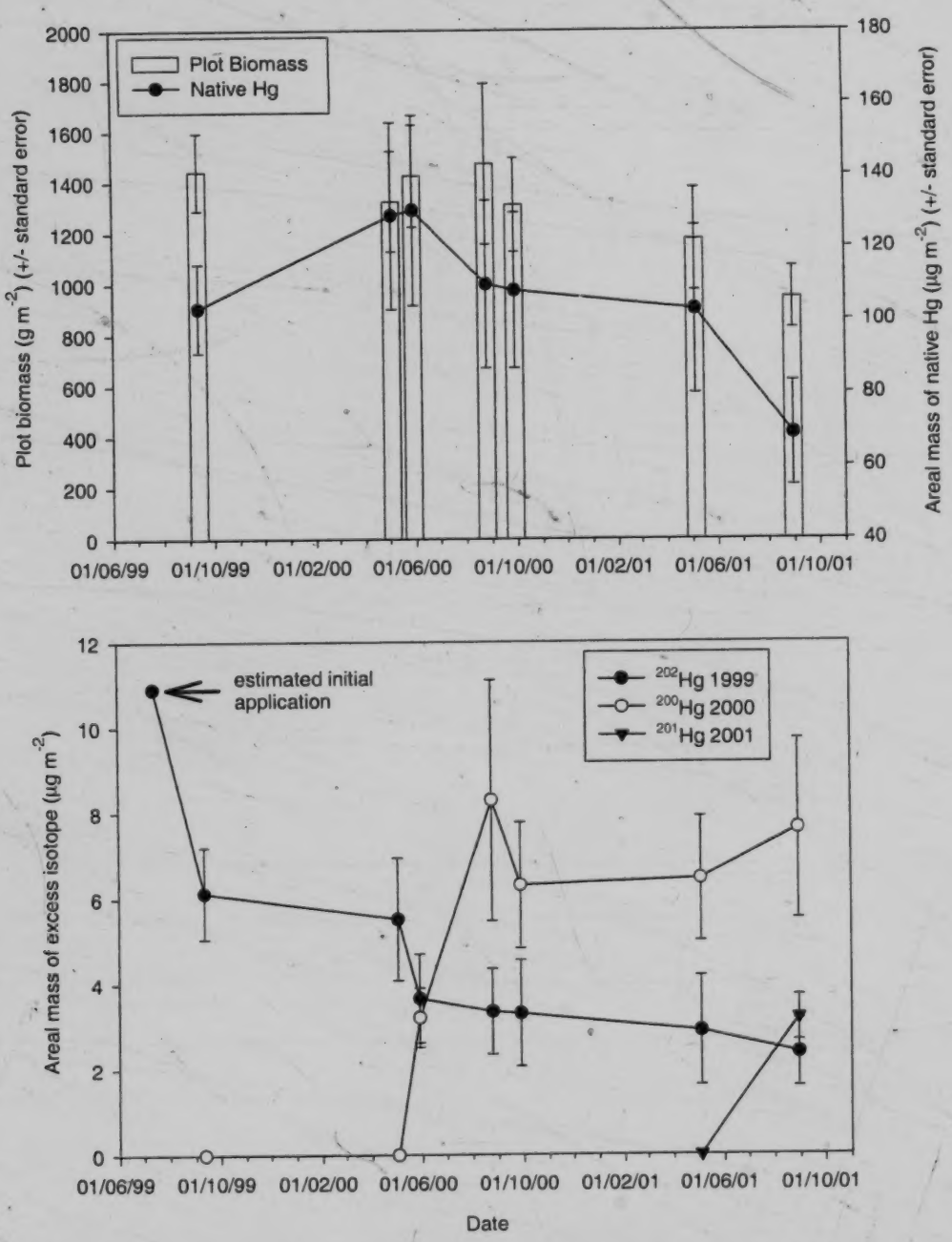

Figure 3.8 Areal biomass, areal mass of native Hg (top panel), and areal mass of ground vegetation-applied Hg isotopes (^{202}Hg, ^{200}Hg, and ^{201}Hg) in U1f from 1999 - 2001. ^{202}Hg was applied in a single application whereas ^{200}Hg and ^{201}Hg were applied monthly.

Discussion

Results from our experiments demonstrate that the majority of the Hg found in boreal canopy foliage is derived from the atmosphere, and not from translocation through roots into foliage. As a result, most of the Hg deposited to the forest floor in litterfall is a new input to watersheds. However, a small portion of the Hg(0) taken up by foliage appears to originate from Hg(0) volatilized from the ground vegetation and soils directly below the canopy. Furthermore, a portion of the Hg wet deposition may bind with foliage and remain there until foliage senescence.

Deposition of atmospheric Hg(0)to foliage: ^{198}Hg permeation tube experiment

Both foliar concentrations of ^{198}Hg and the chamber flux measurement both suggest that there was a deposition of ^{198}Hg(0) to foliage. However, rates of ^{198}Hg(0) deposition estimated by the chamber overestimated by an order of magnitude the amount of ^{198}Hg actually found on the foliage. Estimates of ^{198}Hg(0) deposition to a blank chamber were small (0.087 ng hr^{-1}). This rate of loss to the chamber could account for the discrepancy between the two estimates of ^{198}Hg(0) deposition observed in the first birch run, but is insufficient to explain the observed deposition rates in birch run 2 or the run using jack pine. It is possible that the chamber acted as a larger sink for Hg(0) on some days than others. This is supported by blank chamber runs under field conditions using only native atmospheric Hg(0), in which the chamber acted as a slight sink or source of Hg(0) to varying degrees. While this small chamber effect may be unimportant when measuring large fluxes (such as those seen immediately following the application of simulated rainfall containing Hg^{2+} isotopes (to foliage), it may be extremely important when working at levels near

ambient Hg concentrations in air (as in the permeation tube experiment). For this reason, the tissue concentrations of ^{198}Hg should be considered as a more reliable measure of atmospheric Hg(0) deposition in this experiment.

The presence of atmospheric ^{198}Hg in leaves in this experiment is consistent with the current view that uptake of atmospheric Hg(0) is the major source of the Hg in leaves and litterfall (Mosbaek *et al.*, 1988, Barghigiani *et al.*, 1991, Johnson and Lindberg, 1995, Fleck *et al.*, 1999, Grigal, 2002). Early studies using high concentrations of radioactive $^{203}Hg(0)$ showed that a variety of C3 and C4 plants could accumulate Hg(0) from the atmosphere in their foliage (Browne and Fang, 1978, Du and Fang, 1982). Interestingly, C3 plants (including the majority of the boreal ecoregion species) showed higher rates of Hg(0) uptake than C4 species. However, the high concentrations of Hg(0) used in those experiments (up to 40 $\mu g\ m^{-3}$) created unrealistically high Hg(0) gradients between the atmosphere and the leaves, making extrapolations to a natural forest setting unreasonable. The authors concluded that after entering the leaf during normal gas exchange processes, Hg(0) was bound to a specific site within the leaf and underwent oxidation to Hg(II). As a result, the rate of Hg(0) oxidation to an extent limited the rate of Hg(0) uptake. Unexplained physical and biochemical resistances to Hg(0) uptake were termed mesophyll resistance (Browne and Fang, 1978, Du and Fang, 1982). The importance of mesophyll resistance in limiting Hg(0) deposition was confirmed in a micrometeorological study that modeled Hg(0) exchange within a Tennessee canopy using Hg(0) gradients (Lindberg *et al.*, 1992).

The low levels of excess ^{198}Hg detected in foliage at the end of these experiments, with most observations below the 1% of native LOD, are not surprising considering the short time frame of the experiments (3 hours for birch runs, 6 hours for jack pine run). One

study that followed native Hg content in first year conifer foliage between July and August of one growing season found no significant increase in foliar Hg concentration with time (Rasmussen et al., 1991). However, Hg concentrations in year old needles were higher than in the new growth (Rasmussen et al., 1991). These results implied that at ambient atmospheric concentrations, uptake of Hg(0) from the atmosphere might occur during early growth since there was no increase later in the season. It is possible that we detected uptake of the isotope because our experiments were conducted early in the growing season when leaves were rapidly growing and therefore had high physiological activity. It is interesting to note that absolutely no excess ^{198}Hg was detected in any stem tissues (even if the 1% of native LOD requirement is relaxed), whereas there were traces of ^{198}Hg in all leaf and needle samples. While these foliar concentrations were below the LOD, and therefore must be viewed with caution, they do support the hypothesis that Hg(0) is taken up by foliage via stomata. These results should be clarified with experiments involving longer exposures to the $^{198}Hg(0)$ source.

It is difficult to compare deposition rates observed in this experiment to those published because many of the studies used unnaturally high Hg(0) concentrations in their experiments (Browne and Fang, 1978, Du and Fang, 1982, Hanson *et al.*, 1995). Deposition rates of Hg(0) to canopy foliage in the field are equally difficult to estimate and highly site specific. Ambient atmospheric Hg(0) concentrations vary depending on proximity to point-sources of Hg emission and natural geologic Hg enrichment; atmospheric concentrations are critical in determining the direction and magnitude of foliar Hg exchange (Leonard *et al.*, 1998b).

We observed that the direction of native Hg flux did not necessarily correspond to that of the $^{198}Hg(0)$. In two of the three runs there was an emission of native Hg from foliage while there was a deposition of $^{198}Hg(0)$. Emissions of Hg(0) from plants have been well documented in the literature using both growth chamber (Hanson *et al.*, 1995, Leonard *et al.*, 1998a, Leonard *et al.*, 1998b) and field micrometeorological techniques (Lindberg *et al.*, 1992, Lindberg *et al.*, 2002, in press). Leonard *et al.* (1998a) observed Hg emissions of 10.4 - 92.6 ng m^{-2} hr^{-1} in the light from plants grown in Hg-contaminated soils. Fluxes in the dark were an order of magnitude lower than those in the light, indicating that the dominant pathway of Hg(0) emission was through stomata. In a companion paper, it was determined that the source of foliar Hg(0) emissions was Hg present in the soil, and that Hg(0) emission from foliage only occurred with large changes in soil Hg content (Leonard et al., 1998b). They estimated that plants growing in uncontaminated soils with background native Hg content would have emission rates of 2 - 11 ng m^{-2} hr^{-1}. Our observed emission rates for native Hg were normalized to weight instead of leaf area, and were small. In fact, during the second run on birch leaves we observed a deposition of native Hg(0) to foliage. Native Hg emissions from U1f trees did fall within the range estimated by Leonard et al., (1998b) for foliar emissions in uncontaminated areas (see discussion of U1f results below).

The dual nature of canopy foliage to function as both a sink and source of Hg is well documented (Lindberg *et al.*, 1992, Hanson *et al.*, 1995,). Hanson *et al.*, (1995) observed that at background atmospheric Hg(0) levels, foliage of four different species exhibited mean Hg(0) emissions of 1.7-5.3 ng m^{-2} hr^{-1}. As atmospheric concentrations of Hg(0) were increased, no net exchange was observed, followed by net deposition of Hg(0) to foliage at the highest concentrations. The authors determined that foliar Hg(0) exchange was balanced

around a "compensation point," or an atmospheric Hg(0) concentration below which there is no net Hg exchange, and above which there is a net deposition of Hg(0). This compensation point was observed to be between 10 - 25 ng Hg(0)m^3 in for red maple, Norway spruce, yellow poplar, and white oak. However, this concentration is much higher than that observed at the ELA where we conducted our studies (~1.7 ng m^{-3}), suggesting that Hg(0) compensation points might vary under natural, field conditions. Similar results have been observed in field studies using micrometeorological techniques. Lindberg *et al.*, (1992) showed that emission dominated Hg(0) foliar canopy exchange at the Walker Branch Watershed (Tennessee) on a diel cycle, but that net deposition events were also common at night.

It is clear from these observations that canopy foliage is a dynamic exchange surface with the capacity to function at a given time as either a net sink or a net source of Hg(0). Net emission rates appear to be controlled by factors that have a direct effect on the function of stomata and *P*Hg(0) (e.g., temperature, irradiance) but the relative importance of these environmental factors remains unclear and the subject on ongoing research (Chapter 1, Leonard *et al.*, 1998b).

Uptake of soil Hg by plants: uptake of isotope applied to soils under trees and retention and uptake of isotopes applied to the ground vegetation in a forested micro-catchment

Root uptake of soil-applied ^{202}Hg

The short time between spike application and foliage sampling (~2.5 months) in the ground-applied ^{202}Hg dose response experiment may have contributed to the absence of detectable ^{202}Hg in foliage. This short time frame may have precluded sufficient mobilization of ^{202}Hg from ground vegetation, through soils, and finally into soil water where it would be

available for root uptake. However, the time since dosing should have been less of an issue with trees growing in the U1f microcatchement. The ground vegetation was first dosed with isotope 2 years before the July 2001 sampling date, and there were still no detectable concentrations of excess isotope in the foliage. Hg binds strongly to organic matter in soils (Kim *et al.*, 1997), and release into soil solution may be a very slow process. This potential lag period for atmospherically-derived Hg availability for root uptake is also supported by observations from the U1f microcatchment, where <1% of the ^{202}Hg applied to ground vegetation left the catchment in runoff within the first six months following application. It is likely that the Hg only enters the soil pool following plant senescence and decomposition (Hintelmann *et al.*, 2002). For example, there has been an increasing export of ^{202}Hg in U1f runoff every year post application (Krabbenhoft, pers. comm.). We have continued to collect foliage in 2002 from the dose-response and U1f trees to examine this lag effect.

In addition to the potential time lag associated with ^{202}Hg moving into the soil water, it is possible that the newly-deposited Hg simply does not translocate from soils to foliage and accumulate there at detectable levels. Root uptake is generally not considered a major input pathway for the large amount of Hg found in leaves and litterfall (Grigal, 2002). There is significant evidence from both laboratory and field studies that roots are major binding sites for Hg, and therefore act as a significant barrier for translocation of the metal to foliage. For example, greenhouse studies using solution culture and radiotracer techniques showed that although root uptake and accumulation of radioactive $^{203}HgCl_2$ by *Pisum sativum* and *Mentha spicata* occurred, root Hg content was much greater than that of stems or foliage, and the proportion of Hg retained in roots remained consistent among varying dose levels (~95%) (Beauford *et al.*, 1977). Godbold and Hüttermann (1988) found that spruce seedlings

exposed to a range of Hg concentrations in solution culture showed root Hg concentrations that exceeded those of needles by a factor >100, regardless of the chemical form and external concentration of Hg. The major Hg-binding component of plant cells is the cell wall (Rao *et al.*, 1966, Schönherr and Bukovac, 1970, Beauford *et al.*, 1977) because Hg has a high affinity for fixed (-)ve charges (Beauford *et al.*, 1977).

Studies using Hg contaminated soils show a trend similar to those observed in the solution culture experiments. Bromegrass (*Bromus inermis*) grown in undisturbed soil columns and treated with mercuric chloride had much higher concentrations of Hg in roots than in foliage, and fine roots had 2-4 times the Hg concentration of primary and secondary roots (Hogg *et al.*, 1978). Stem Hg concentrations were intermediate between those of roots and foliage, indicating that only a small amount of the Hg absorbed from roots actually accumulated in the foliage. Lindberg *et al.* (1979) showed using alfalfa (*Medicago sativa*) grown in contaminated surface soils collected near the Almadén Hg mine (Spain) that roots accumulated Hg in proportion to soil concentrations, but Hg in above-ground tissues was not derived from root uptake. Hg concentrations in the roots of the alfalfa grown in contaminated soils were 4 - 5 times higher than in above-ground material whereas Hg concentrations in roots of control plants were three times lower than in above-ground parts. Plants grown in both contaminated and control soils had similar above-ground Hg concentrations. Direct absorption of Hg vapor by leaves was thought to responsible for the majority of Hg accumulation in above ground tissues (Lindberg *et al.*, 1979).

Several studies have shown that plants grown in Hg-contaminated soils have a hierarchy of Hg concentrations as follows: soil >> roots > stems ≥ leaves, and that Hg transfer coefficients increase acropetally (Talmage and Walton, 1993, Cocking *et al.*, 1995,

Leonard *et al.*, 1998a). Leonard *et al.*, (1998a) found that soil Hg concentrations exceeded those in roots by at least an order of magnitude, and those in above-ground tissues by at least two orders of magnitude. While a very small amount of soil Hg actually entered roots, the proportion of Hg transferred upward increased substantially (by at least an order of magnitude) at each step (roots → stems → leaves).

All these studies suggest that while root uptake of soil Hg may and sometimes does occur, this uptake mechanism cannot account for the concentrations of Hg in foliage and litterfall. In fact, when transport of Hg in xylem sap of Scots pine and Norway spruce was investigated, it was concluded that at most 11% of the Hg in litterfall could have originated from root uptake (Bishop *et al.*, 1998).

Native Hg concentrations in canopy foliage

Hg concentrations in tree foliage in this study are near the low end of the range reported for canopy foliage in uncontaminated areas (10 - 300 ng Hg g^{-1}, Moore *et al.*, 1995). Foliar concentrations in polluted areas can exceed 10 000 ng g^{-1}. (Moore *et al.*, 1995). Hg concentrations in leaves of trees (*Picea mariana, Pinus banksiana, Betula papyrifera, Larix laricina*) and shrubs (*Chaemedaphne calyculu, Ledum groenlandicum, Alnus rugosa*) previously collected at the ELA ranged from 5 - 23 ng g^{-1} (Moore *et al.*, 1995). Black spruce needles collected from a wetland at the ELA (28 ng g^{-1}, Heyes *et al.*, 1998) and from Quebec (23 - 34 ng g^{-1}, Zhang et al., 1995) had comparable Hg concentrations to those reported here. Foliar concentrations of Hg in this study are also similar to those reported for balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*) in northern Ontario (Rasmussen, 1995). The low concentrations of Hg in foliage at the ELA are consistent with the fact that this area is far from any point-sources of contamination. Annual wet deposition of Hg at the ELA is low (71 mg ha^{-1} yr^{-1}) (St.Louis

et al., 2001) compared to other regions like Southern Sweden (100 - 350 mg ha^{-1} yr^{-1} Hg in wet deposition) (St.Louis *et al.*, 1995).

The significantly higher concentrations of Hg in year-old needles of jack pine and black spruce compared to new growth in this study is consistent with the observation that Hg content of leaves/needles of many species increase over the course of the growing season (Rasmussen *et al.*, 1991, Rasmussen, 1995, Bombosch, 1983, Barghigiani *et al.*, 1991, Wyttenbach and Tobler, 1988, Fleck *et al.*, 1999). Rasmussen, (1995) found that the concentrations of Hg in northern Ontario balsam fir and white spruce needles more than doubled within each growing season. This temporal variation in foliar Hg concentration was most pronounced for new growth, and older tissues increased by 5 - 10 ng g^{-1} yr^{-1}. Similar increases in the concentration of Hg in Norway spruce (*Picea abies*) needles were observed over the course of the growing season in Switzerland (Wyttenbach and Tobler, 1988). Again, accumulation of Hg proceeded faster in the first growing season than in the second. Fleck *et al.* (1999) observed twice as much Hg in 2-year old needles of red pine (*Pinus resinosa*, 16.8 - 28.3 ng g^{-1}) than was present in year-old needles (8.3 - 17.0 ng g^{-1}). In their study, needle Hg concentrations were closely related to length of the growing season (and hence duration of physiological activity), but not correlated to soil Hg concentration, leading the authors to conclude that foliar uptake of atmospheric Hg(0) contributed to needle Hg concentrations.

We observed significantly higher concentrations of Hg in needles of both black spruce and jack pine collected at a height of 1 m than at 3 m. This is contrary to findings by Rasmusssen, *et al.*, (1991), who found that height above ground did not have a significant effect on needle Hg concentrations. However, other studies suggest that plants of lower stature generally tend to have greater concentrations of Hg than trees at the same location

(Rasmussen, 1994). Moore *et al.* (1995) showed that when plants and fungi were placed in ecological groupings, they showed the following trend in Hg concentrations: grasses and herbs < tree and shrub leaves < aquatic macrophytes < *Sphagnum* mosses < lichens< fungi. Hg content was greater in non-vascular than in vascular plants, and tended to increase with increasing proximity of the plant's habitat to the water table. The trend of taller, vascular plants having lower Hg concentrations may result from exposure to lower atmospheric Hg(0) near the top of the canopy compared to near the forest floor. Lindberg *et al.*, (1992) showed gradients of decreasing Hg(0) with height within and above the forest canopy, presumably originating from soil Hg(0) emissions (Walker Branch watershed, Oak ridge, Tennessee). If higher foliage is exposed to less Hg(0) on a continued basis, and stomatal uptake of atmospheric Hg is the main source of foliar Hg, this could explain why we observed higher concentrations of Hg in needles closer to the ground.

Ambient and isotopic fluxes from black spruce and jack pine in U1f

We observed no detectable levels of soil-applied ^{200}Hg or ^{202}Hg in the Hg(0) emissions from U1f trees. This is surprising, considering that the likely source of Hg(0) emitted from foliage is soil Hg transported there via the transpiration stream. Leonard *et al.*, (1998a) developed a 2-compartment model of Hg transport within plants. This model predicts that there are not just static pools of Hg in tissues (which change in concentration on the time scale of days to weeks), but also dynamic transport of Hg(0) from the soil, through the transpiration stream, and out the stomata that occurs on the order of minutes to hours. Leonard *et al.*, (1998a) found that for every molecule of Hg that occurred in leaf tissue, 12 were volatilized from the mesophyll and released via stomata. This indicates that far more Hg passes through plant canopies than is actually stored in foliage. Although we

had expected to see the soil-applied isotopes in U1f foliar Hg(0) emissions based on this model, the lack thereof is consistent with the absence of any excess isotopes in the needles themselves. Again, it is likely that there has been insufficient time for the applied isotopic Hg to be mobilized from live vegetation through the decomposition process and into soil water pools. Measurement of fluxes from U1f vegetation and tissue sampling should continue over the long term. This is especially important because concentrations of spike in U1f runoff have continued to increase over time. It is possible that increased concentrations of ^{202}Hg and ^{200}Hg in soil-water will eventually result in detectable fluxes of the isotopes from foliage.

The average native Hg emissions we measured here from jack pine (6.7 ng m^{-2} hr^{-1}) and black spruce (2.4 ng m^{-2} hr^{-1}) are within the range estimated by Leonard *et al.*, (1998b) for plants growing in uncontaminated areas (2-11 ng m^{-2} hr^{-1}). The higher Hg(0) emissions from jack pine might be explained by the apparent high transpiration rate of the jack pine used in this experiment. In the short amount of time between the placement of the jack pine branch in the chamber, and initiation of the flushing pumps, there was a slight build-up of humidity in the chamber that was rapidly dispersed with a few moments flushing. No build-up of humidity occurred in the chamber containing the black spruce. If the source of foliar Hg(0) emissions is the soil-water Hg(0) pool (via the transpiration stream), greater observed rates of native Hg evasion from jack pine could be in part explained by greater transpiration rates. Unfortunately, no measurements of transpiration are available for these runs.

U1f ground vegetation

Concentrations of ^{202}Hg in U1f ground vegetation decreased over time, in a trend similar to that seen on trees sprayed with ^{202}Hg in simulated rainfall. Rates of Hg loss decreased over time, and concentrations on the vegetation plateaued around 3 μg m^{-2} for the

last four reported sampling dates in 2000 - 2001. Reduction of Hg(II) to Hg(0), and subsequent volatilization from ground vegetation to the atmosphere, may be responsible for some of the loss of the isotope. Initial (13 July 1999) flux measurements made by S. Lindberg (ORNL, Tennessee) using polycarbonate flux chambers were 4 and 7 ng m^{-2} hr^{-1} over blueberry and moss-covered soils, respectively (Hintelmann *et al.*, 2002). These volatilization rates decreased to <0.1 ng m^{-2} hr^{-1} by 26 October 1999. It was estimated that only between 0.3-1 mg of the total 7.7 mg ^{202}Hg applied was lost via volatilization in the first 3 months post application, compared with 0.01-0.04 mg lost via runoff. The bulk of the spike remained attached to the vegetation (3.7-5.1 mg), and some entered the soil pool (1.6-3.7 mg) (Hintelmann *et al.*, 2002). It is likely that the ^{202}Hg that remained on vegetation was slowly lost from foliage through senescence of vegetation and litterfall. This is consistent with the observation by Hintelmann *et al.*, (2002) that newly deposited Hg in dry deposition or small rain events rapidly equilibrates with the native Hg pools and that the small portion not initially volatilized or lost in runoff becomes fairly immobile. This conclusion is supported by the behavior of the 2000 spike (^{200}Hg). This isotope was applied in monthly applications, and did not exhibit the rapid decline followed by a leveling-off in areal mass seen with the 1999 isotope. Instead, this second isotope increased and plateaued at an areal mass of ~6 - 8 mg ^{200}Hg m^{-2}. Even by the last sampling date in 2001, the areal mass of ^{200}Hg in ground vegetation had not started to decrease, suggesting that more frequent, smaller applications resulted in greater retention of the Hg isotope. It appears that the 2001 spike (^{201}Hg; also applied in monthly applications) initially behaved in a similar fashion. Continued, long term sampling of both ground vegetation, soil, and runoff will clarify how much of

newly deposited Hg reaches soil and soil-water pools, and the time scales necessary for this transfer.

Changes in areal mass of native Hg in ground vegetation were not similar to those for the isotopes. Areal mass of native Hg was fairly constant over time, ranging between ~70 - 130 $\mu g\ m^{-2}$ amongst all sites. Native Hg concentrations in ground vegetation were comparable to published values from the same area (Ontario, precambrian shield). Rasmussen (1994) reported a range of lichen (*Cladina* spp. and *Cladonia* spp.) Hg concentrations between 17.2 - 66.9 $ng\ g^{-1}$. Moore *et al.*, (1995) reported mean lichen Hg concentration at the ELA of 31.9 $ng\ g^{-1}$. Pleurocarpus mosses accumulate significant amounts of native Hg, and ground-growing *Pleurozium schreberi* concentrations are reported to range from 35.1 - 286.9 $ng\ g^{-1}$. ELA feather mosses contain an average of 80.3 $ng\ g^{-1}$ native Hg (Moore *et al.*, 1995). Bryophytes are known to be accumulators of many metals, and are often used as biomonitors of heavy metal pollution. No published values are available for blueberry, however other trees and shrubs at the ELA have low concentrations of native Hg (average 14.2 ng g-1) (Moore *et al.*, 1995). Many of the U1f quadrats contained a mixture of shrubs, lichens, and mosses, and the range of native Hg concentrations in these quadrats were consistent with reported Hg concentrations measured in their constituent vegetation types (Moore *et al.*, 1995, Rasmussen, 1994).

Conclusion

Our experiments are consistent with the view that the majority of the Hg is a new input of Hg to watersheds. It appears that the source of Hg in leaves is more likely derived from the atmosphere than from root uptake of soil-water Hg. It is possible that some portion of the Hg in leaves is derived from uptake of volatilized Hg from subtending soils, based on the discrepancy between Hg concentrations of high and low foliage of U1f trees. However, this possibility was not confirmed by presence of any ground-applied isotopes, and requires more investigation. A proportion of the Hg in leaves may also be derived from retention of Hg(II) from wet deposition.

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Chapter 4 Mercury, the forest canopy and METAALICUS

General Discussion

The general goal of my research was to investigate the source of mercury (Hg) in boreal forest canopy foliage and litterfall. Litterfall represents the largest input of Hg to the forest floor, and knowing the source(s) of this Hg is crucial for understanding the net deposition of Hg to ecosystems (St.Louis *et al.*, 2002).

In chapter 2, I presented the design and testing of a new dynamic chamber system for measuring Hg(0) exchanges from canopy vegetation in uncontaminated areas. The chamber design addressed for the first time several problems associated with previous chamber systems that may have influenced measured Hg(0) fluxes from foliage. These factors included low transmission of visible and UV radiation wavelengths, low mixing of the internal environment and air turnover, and disregard of normal physiological functioning of enclosed vegetation. When fluxes of ^{202}Hg(0) from foliage sprayed with ^{202}Hg(II) were compared with isotope loss rates derived from actual foliar concentrations, it was determined that either the chamber measurements underestimated Hg(II) reduction and volatilization, or there was another mechanism of Hg loss occurring, such as weathering of cuticles. I also determined that solar radiation (both visible and UV wavelengths) had a strong influence on reduction and volatilization of newly deposited Hg(II) on foliage.

Chapter 3 presented mechanistic experiments that addressed three potential pathways of Hg uptake by boreal forest plants: 1) stomatal uptake, 2) root uptake, and 3) retention of Hg(II) deposited in wet deposition. Results were consistent with the general views that the majority of Hg in litterfall is derived from stomatal uptake of atmospheric Hg(0), and that root uptake and translocation is not a major contributor to Hg accumulation

in leaves. An unexpected conclusion from both chapters was that some of the Hg(II) applied to vegetation in simulated wet deposition was retained over time, suggesting that foliage may not just act as a source for Hg in throughfall, but may also retain some of the newly deposited Hg over time.

Future research should continue to evaluate the role of Hg exchange in the canopy of forests. This type of research constitutes our primary focus in the unique, whole-ecosystem experiment called the *Mercury Experiment to Assess Atmospheric Loadings in Canada and the U.S.* (METAALICUS) designed to examine the mobility and availability of newly deposited atmospheric Hg.

METAALICUS

The general concensus among Hg scientists is that elevated atmospheric Hg inputs to watersheds are the primary cause of high concentrations of monomethyl mercury (MMHg) in fish (Hintelmann *et al.*, 2002). In hopes of protecting public health, governments have proposed controlling industrial Hg emissions with a projected cost of billions of dollars. To evaluate the effectiveness of these proposed Hg emissions controls, an understanding of the effect of reduced atmospheric Hg loading on fish MMHg levels is crucial. Previous research has been unable to determine whether these controls will be sufficient, overprotective, or have no effect on MMHg levels in fish. This is because newly deposited atmospheric Hg is indistinguishable from background pools of Hg, accumulated over time in different compartments of the ecosystem, using standard Hg measurement techniques (e.g., cold vapor atomic fluorescence spectrometry). By using stable Hg isotopes as tracers and inductively coupled plasma mass spectrometry detection techniques,

METAALICUS scientists can distinguish newly deposited isotopic Hg loaded onto the ecosystem from background, native pools of Hg. Three different isotopes have been added to the upland, wetland, and lake surface of the L658 watershed at the Experimental Lakes Area (ELA), northwestern Ontario, in the years 2001 and 2002 (Figure 4.1). Experimental loading of Hg increased the atmospheric load to the watershed by 4-5 times, to a deposition rate equivalent to those observed in the Northeastern US (METAALICUS, 2002) (Figure 4.2). Ongoing comprehensive field studies are being carried out to quantify Hg isotope concentrations in all compartments of the watershed, and to monitor how they change with time. The upland and wetland isotopes are applied to the canopy by crop duster aircraft (Figure 4.3), and necessary ambient conditions for spike application were determined in part by our preliminary findings in the previous two chapters. The observed effect of solar radiation on $^{202}Hg(0)$ evasion from foliage dictated that the spikes be applied under cloudy, rainy conditions. This helped to both minimize volatilization, and to attain maximum penetration of the spike through the canopy to the forest floor. Our continued work on the METAALICUS project involves collection and analysis of upland and wetland canopy foliage, litterfall, throughfall, and ground vegetation, in hopes of generating a terrestrial mass-balance for the applied isotopes.

The integrated approach of combining mechanistic and microcatchment scale experiments (e.g., U1f) with large, whole-ecosystem experiments like METAALICUS should provide the most complete picture possible of the biogeochemical cycling of Hg in boreal forest canopies and entire watersheds. In addition, insights generated from these experiments will finally allow predictions to be made in terms of the effectiveness of reducing the industrial Hg emissions on MMHg concentrations in fish.

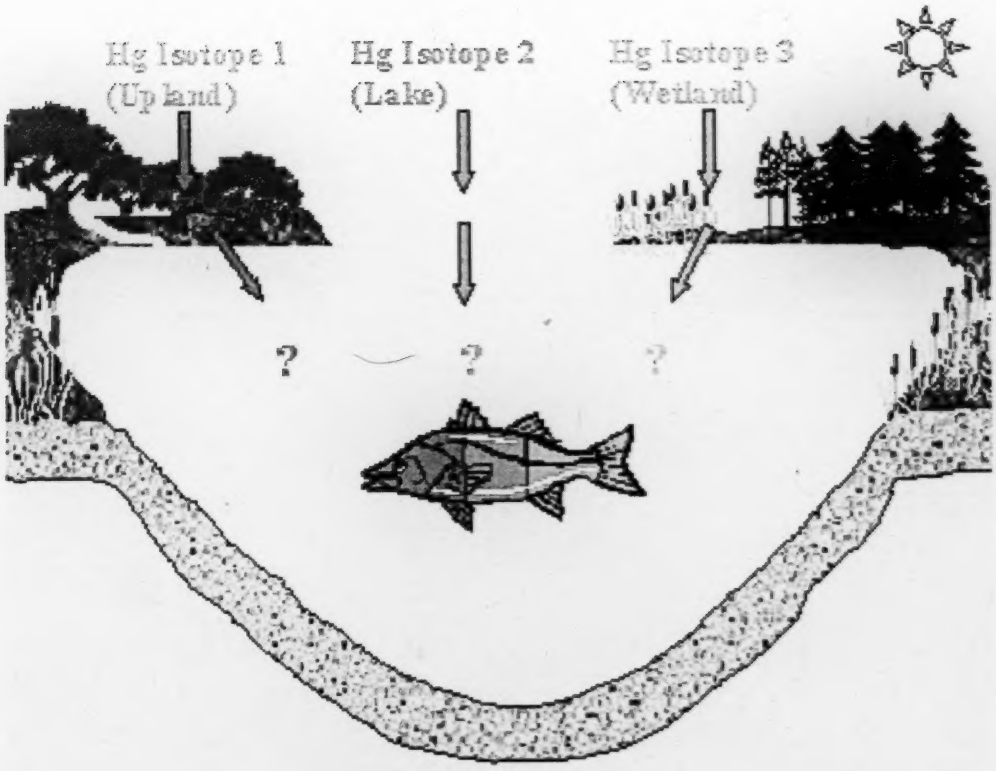

Figure 4.1 Use of stable Hg isotopes to evaluate how route of entry (upland runoff, direct deposition, or wetland outflow) affects Hg accumulation in fish (METAALICUS, 2002).

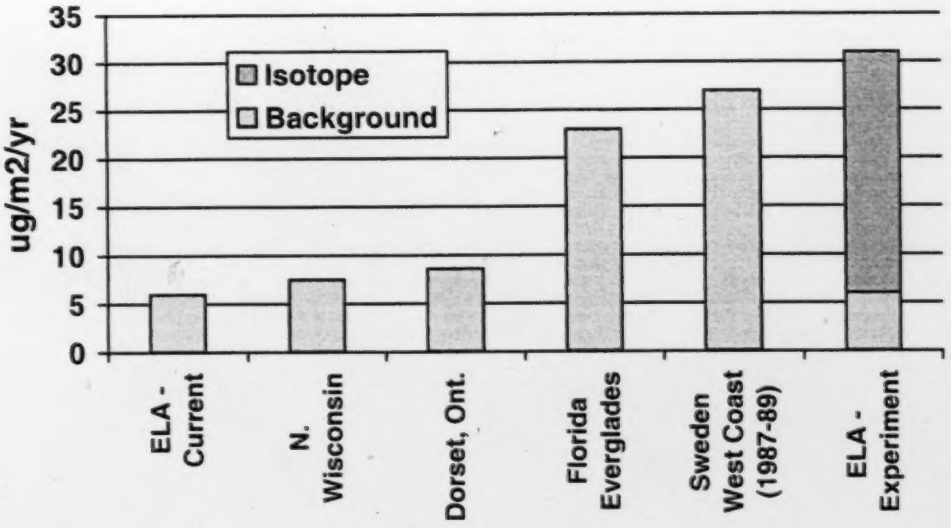

Figure 4.2 Proposed loading rates of stable Hg isotopes in the METAALICUS project (METAALICUS, 2002).

Figure 4.3: Aerial application of Hg to L658 upland, June 2001 (METAALICUS, 2002).

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